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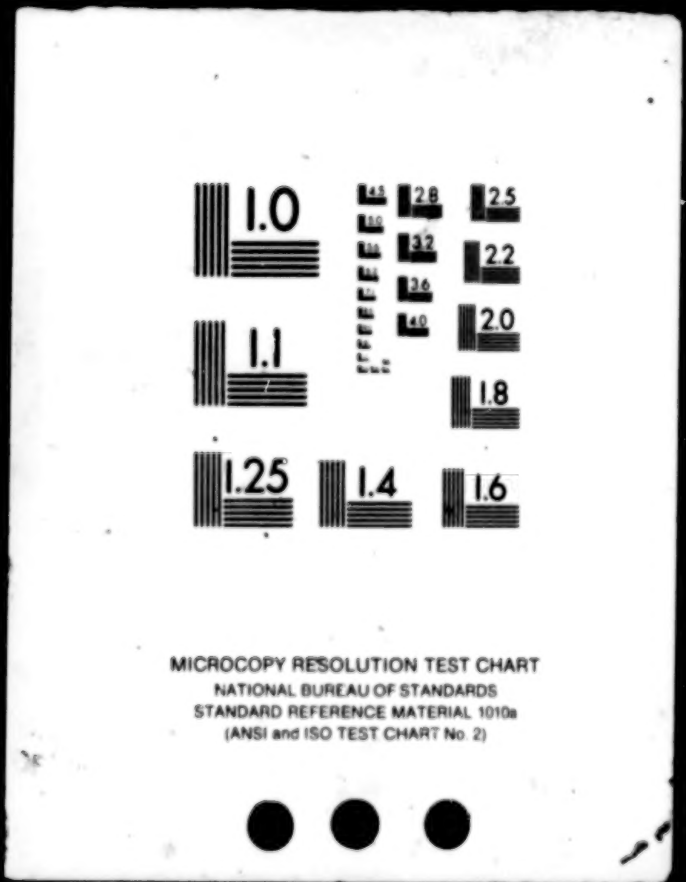

NASA Conference Publication 2480

Controlled Ecological Life Support System

Regenerative Life Support System in Space

Robert D. MacElroy and David T. Smernoff

(NASA-CP-2480) CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEM: REGENERATIVE LIFE SUPPORT SYSTEMS IN SPACE (NASA) 153 p Avail: NTIS HC A08/MF A01 CSCL 06K
N88-12251 --THRU-- N88-12273
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H1/54 0106412

Topical session of the
XXVI COSPAR meeting
Toulouse, France
July 1986

NASA

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Regenerative Life Support System in Space

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Topical session of the
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National Aeronautics
and Space Administration
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Moffett Field, California 94035

1987

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PREFACE

Reliable and efficient methods of life support must be developed if human crews are to survive for long periods in space and far removed from Earth. Methods of life support based either on the resupply or physical-chemical regeneration of materials are limited to small crews and short mission durations. Such methods are appropriate for Shuttle and Space Station, but for the establishment of bases on the Moon or Mars, or transits to the outer planets alternate approaches are needed. *Bio*regeneration of materials can supply food, oxygen and water and remove all waste materials from the crew's environment.

The President of the United States recently commissioned the National Commission on Space to propose a long term program for the National Aeronautics and Space Administration. The commissions report was released in April of 1986, and states specifically that the development of closed ecology life support technology should be actively pursued by NASA for it to be usable for the space missions anticipated during the latter part of this century and beginning of the next. Interest in such bioregenerative life support systems is not limited to the United States; European, Canadian and Japanese research in this area is being initiated, and the Soviet Union has had a very active and productive program in this area for many years.

Twenty-two papers were presented in Workshop II of the XXVIth COSPAR meeting ("Regenerative Life Support Systems in Space"), which was held in Toulouse, France during July 1986. The papers cover a wide variety of topics related to the extended support of humans in space. Several papers deal with overviews of research conducted in Japan, Europe and the United States. Many papers are concerned with the methods and technologies required to recycle materials, especially respiratory gases, within a closed system. There are also papers which address issues related to plant and algal productivity, efficiency and processing methods. Computer simulation of closed systems, discussions of radiation effects on system stability, and modelling of a complete bioregenerative system were also presented.

These papers represent many of the issues associated with the development and operation of bioregenerative life support systems, or in NASA terminology, Controlled Ecological Life Support Systems (CELSS). Individually they depict the range of thought that is necessary for the successful development of an 'ecological' system that is designed to support a crew, and to function stably for long periods. Without the forum provided by the COSPAR meeting the international mix of scientists and engineers would not be possible. It is anticipated that such international cooperation will continue and significantly enhance the development of a CELSS.

Robert D. MacElroy
David T. Smernoff

November 21, 1986

N88-12252

PROGRESS IN EUROPEAN CELSS ACTIVITIES

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ABSTRACT

The European CELSS* activities started in the late 1970's with system analysis and feasibility studies of Biological Life Support Systems (BLSS). Since then the European efforts have continued in two major directions: as a series of individual development tasks like the Environmental Life Support System and the Solar Plant Growth Facility, and in parallel hereto as overall coordination and planning activities for life support system long term needs definition and payload definition for COLUMBUS utilization.

The early initiations for CELSS came from the industry side in Europe, but since then planning and hardware feasibility analyses have been initiated also from customer/agency side. Despite this, it is still to early to state that a 'CELSS- programme' as a 'concerted' effort has been agreed upon in Europe. However, the general CELSS objectives have been accpeted as planning and possible development goals for the European effort for manned space activities, and as experimental planning topics in the life sciences community for the next decades.

INTRODUCTION

At the end of the 1970's the European Space industry was faced with the immediate completion of the development and the soon to come delivery of the first SPACELAB flight unit. In this situation the future medium and long term development scenarios for life support systems were analysed, which resulted in conceptual studies and experimental development work for regenerative physio-chemical life support system hardware and feasibility studies for closed life support systems.

The Biological Life Support System (BLSS) study undertaken by Dornier System in cooperation with Hamilton Standard had as a goal to analyse the feasibility of ecological life support system for space applications and to define problem areas requiring immediate attention. The study thus resulted in a proposed programme for the develoment of a BLSS as an international and multidisciplinary cooperative effort /1/.

Since the start of the COLUMBUS programme the planning for a long term manned space scenario has been performed by several European space agencies (e.g. the European Space Agency, ESA, and the German Aerospace Agency, DFVLR) in order to define key technology issues and a coordinated long term technology development programme beyond year 2000.

Two hardware oriented projects with testing of small scale ecological life support systems have been pursued through the bread-board testing and could be candidates for experimental flight hardware in the COLUMBUS programme.

LONG TERM NEEDS

With the official time period for long term goals to cover the next 30 years, the envisaged manned space mission scenario covers both long duration missions (several years) and larger permanently manned space bases (20-50 or more persons) in most European ongoing studies.

This has resulted in a tentative long term technology development programme for life support systems with the closure of the carbon loop as the ultimate goal. The necessary technology issues to be dealt with in order to achieve the implementation of these controlled ecological or biological life support systems have been analysed.

---

* Controlled Ecological Life Support System, CELSS

Tasks of immediate importance from a life support system development point-of-view are:

- investigations concerning plants in micro-gravity,
- investigations concerning the cosmic radiation environment and protection,
- development of appropriate illumination concepts for an optimized overall energy balance,
- monitoring, control and sensor technology for biological systems,
- cultivation and havesting methods in micro-gravity, and
- biological waste processing.

It can be expected that very soon the controlled ecological life support system development will become one of the final goals in several international and national long term space planning documents in Europe.

COLUMBUS UTILIZATION

The European Space Agency, ESA, has performed a series of studies on 'European' Utilisation Aspects of Low Earth Orbit Space Station Elements (EUA)' in parallel to the COLUMBUS space station system design work /2/.

A total of 17 model payloads for the space station attached Pressurized Module and the Platforms have been established and investigated in detail. Three of these payloads contain defined CELSS objectives:

- General Purpose Life Science Research Lab.
  LIF 111, start of operations at COLUMBUS IOC.
  Preliminary investigation in CELSS.
- Exo- and Radiation Biology on Co-orbiting Platform.
  LIF 311, start of operations at COLUMBUS IOC.
  Biological experiments for ecological life support system.
- Production Bioprocessing.
  LIF 312, start of operations of COLUMBUS IOC plus ~ 3 years.
  Fully automated production facilities for bio-processing and biological CELSS on a Co-orbiting Platform (engineering phase of ecological life support system development).

Similar objectives are also contained in the German Microgravity Research Programme, where interests in basic research for the development of future ecological (biological) life support systems are defined.

HARDWARE DEVELOPMENT

Presently two European projects directly associated with the development of CELSS are in the bread-board phase:

- Solar Plant Growth Facility (SPGF), and
- Environmental Life Support System Study (ELSS).

Solar Plant Growth Facility

The SPGF project is a cooperative Austrian-German effort for the development of a reusable life science facility for investigations with respect to future ecological (biological) life support systems /3/.

Technically the SPGF (Figure 1) will be used to verify handling and cultivation methods for larger amounts of biological material necessary for food production and atmosphere revitaligation.

A bread-board model has been built and is presently used for various types of plant experiments with the aim to give more data on handling and function for design improvement. The size of this system (660 x 1360 x 900 mm) is large than most other comparable planed units, which would allow for experimenting with larger plants and technically complex support equipment like window and shutter for illumination investigation and stem-cutting concept harvesting hardware.

The continuation of this project is presently undetermined.

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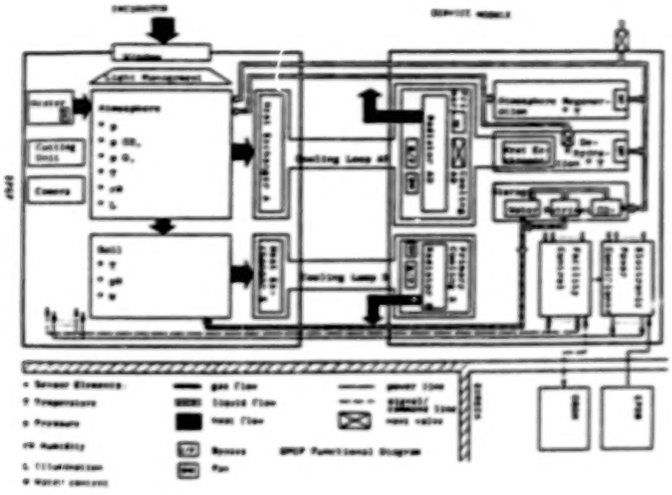


Fig. 1. Solar Plant Growth Facility, SPGF.

Environmental Life Support System

The major goal of the ELSS-project is to develop a support system for biological experiments in space. This support (atmosphere supply, water supply, food and waste management, and thermal control) is intended to be performed as far as possible by biological means /3/.

It is seen also as a test bed for CELSS. The present state of the design includes concepts for (Figure 2):

- CO_2 conversion into oxygen,
- recycling of organic wastes (partly),
- supply of nutrients

by biological means.

Water recirculation and thermal conditioning are performed via physical methods. CO_2 is delivered from a gas bottle, because it is primarily for the build up of biomass in the experiments. For the conversion of CO_2 into O_2 a unicellular alga is used, which allows also food to be supplied by excretion of carbohydrates and recycling of organic waste (e.g. urea).

A bread-board model has been built and is presently under testing to prove the design of the biological part of the system.

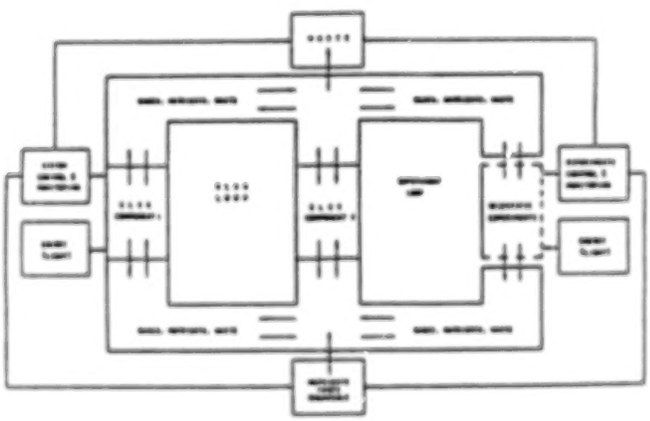


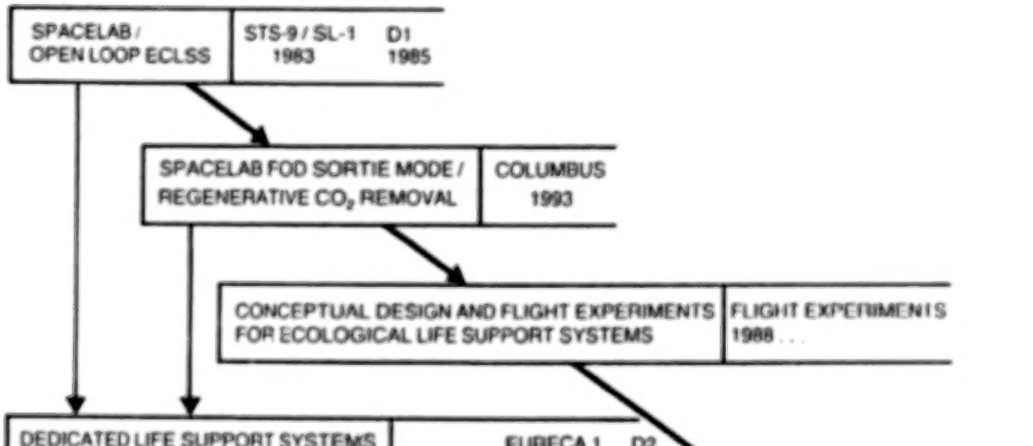
Fig. 2. Environmental Life Support System, ELSS.

CONCLUSIONS

After initial industrial studies and feasibility analysis concerning ecological life support systems the general European situation and the attitude of various space agencies has begun to change lately towards accepting CELSS as a long term development goal. That is, planning and definition of scientific and technological tasks for CELSS are opportune and part of several ongoing studies in Europe.

It is expected that ecological life support systems can be tested and implemented on a space station towards the end of this century or early in the next. For the European activities a possible scenario can be projected based on ongoing life support system development activities and the present life sciences goals (Figure 3).

Europe has a strong position in many of the scientific and technical disciplines relevant to CELSS development activities and could become an important partner in a future very large and challenging development of ecological life support systems. The multitude of tasks makes the CELSS work most suited for an international cooperation.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STS-9 / SL-1 1983
D1 1985
SPACELAB FOD SORTIE MODE / REGENERATIVE CO_2 REMOVAL
COLUMBUS 1993
CONCEPTUAL DESIGN AND FLIGHT EXPERIMENTS FOR ECOLOGICAL LIFE SUPPORT SYSTEMS
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DEDICATED LIFE SUPPORT SYSTEMS FOR LIFE SCIENCES EXPERIMENTS
EURECA 1 1987
D2 1988
BUILD-UP OF ECOLOGICAL LIFE SUPPORT SYSTEMS AND MANNED EVALUATION
FLIGHT 1990...

Fig. 3. Anticipated CELSS development logic in Europe. (Note: Implications of a new Shuttle flight manifest have not been considered).

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N88-12253

FOOD PRODUCTION and GAS EXCHANGE SYSTEM using BLUE-GREEN ALGA (SPIRULINA) for CELSS

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ABSTRACT

In order to reduce the cultivation area required for the growth of higher plants in space adoption of algae, which have a higher photosynthetic ability, seems very suitable for obtaining oxygen and food as a useful source of high quality protein.

The preliminary cultivation experiment for determining optimum cultivation conditions and for obtaining the critical design parameters of the cultivator itself has been conducted.

Spirulina was cultivated in the 6-liter medium containing a sodium hydrogen carbonate solution and a cultivation temperature controlled using a thermostat. Generated oxygen gas was separated using a polypropyrene porous hollow fiber membrane module. Through this experiment, oxygen gas (at a concentration of more than 40%) at a rate of 100 ~ 150 ml per minute could be obtained.

INTRODUCTION

To support the long duration stay of human beings in space, technology to recover O_2 gas from CO_2 gas exhaled by human beings and a continuous food production system must be established. Environmental control system hardware for supporting life science experiments plans to use solid amine for removing excessive CO_2 gas and some kind of salcomine for recovering excessive O_2 gas produced by higher plants and algae. Otherwise, Sabatier or Bosch method is also planned to be used for reduction from CO_2 gas to O_2 gas. Though these physico-chemical methods are very effective for gas exchanges, they have no ability to producing foods.

The utilization of biological methods in space is advantageous for producing food and also for conducting gas exchange simultaneously. However, there are various problems with this method. If we plan to conduct the gas exchanges using only higher plants a very large cultivation area is required. The required quantity of O_2 gas per day for supporting a crew is about 0.9 kg. Harvesting of 1.0 kg dry weight of leaf vegetables such as lettuce through about 3 month cultivation time requires about 1.6 kg of CO_2 gas and produces 1.2 kg of O_2 gas. To get 0.9 kg of O_2 gas, for example, cultivation area where produces 70 k_4 of lettuce is required. Therefore, another bio-species such as algae having higher photosynthetic ability is profitable to be adopted in Controlled Ecological Life Support Systems (CELSS).

UTILIZATION OF ALGAE AS FOOD RESOURCE

To use algae as food, it is desirable to establish the following conditions; ① Mass production ability. ② High quality protein and mineral biomass. ③ Lower production costs. ④ No toxicity. ⑤ Acceptable taste.

Chlorella, a green alga, has been studied for about 30 years and could be used as the protein source, but utilization of Chlorella as food in CELSS seems very difficult for following reasons; ① A high speed centrifugal separator which needs very high energy consumption must be used for harvesting because of its very small size (nearly 5 μm). ② Since the cell wall consists of crude fiber, it is indigestible by the human body without pulverization. ③ Contamination by a virus or a bacillus occurs easily because the nutrient solution is weakly acidic.

Then, Spirulina, a kind of blue-green alga, has been proposed because of the following distinctive features; ① Harvesting is very easy because the size is about 100 times that of Chlorella. ② Cell is made of viscous polysaccharide and is very easily digested. ③ Contamination seldom occures because the medium is heavily alkaline.

These characteristics cover most of the weak points of Chlorella and furthermore, the protein contained in Spirulina is high quality. About 30 kinds of Spirulina have been found, but only several species among these have good properties with high protein, no toxicity, high propagating ability and big size for harvesting. Especially, Spirulina platensis and Spirulina maxima are the well-known species produced in industrial scale. Since the range of the suitable cultivation temperature for Spirulina is from 30 to 40 ℃, the waste heat from other facilities in CELSS can be used.

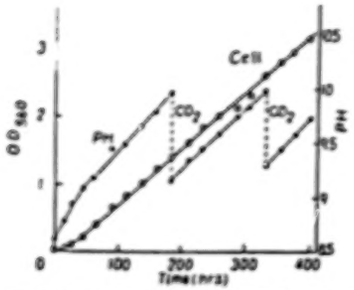
As shown in Fig. 1b the alkaline medium, range of its pH is from 9 to 12, has a very high utilization efficiency of CO_2 [1]. Spirulina utilizes sodium hydrogen carbonate ($NaHCO_3$) as the source of CO_2 necessary for photosynthetic reactions. $NaHCO_3$ is dissociated into HCO_3^- or CO_3^{2-} in the solution as shown in Fig. 1. When the photosynthetic reaction of Spirulina proceeds, the concentration of CO_3^{2-} increases relatively. The rise of pH value as shown in Fig. 1a results from the progress of this reaction [2]. To maintain continuous cultivation, pH value must be held within some range (i.e. 9~10). If pH value goes over the limit value, CO_2 must be provided in the culture solution to reduce pH value. These operations are shown as the discontinuous broken lines of pH in Fig. 1a. On the other hand, since the medium for Chlorella is weakly acidic, solubility of CO_2 are not so high, therefore, it is very difficult to increase the concentration of CO_2 in the culture solution. To use Spirulina in CELSS seems more suitable than Chlorella from comparing these characteristics of Chlorella and Spirulina.

NUTRITIVE VALUES AND PRACTICAL USE OF SPIRULINA IN CELSS

The nutritive values and components of Spirulina are very important factors to use it as food. The organic components of Spirulina have the following distinctive features;
① Abundant protein content. ② Carbohydrate consists of glycogen (so called animal starch). ③ Containing linoleic acid and linolenic acid (unsaturated fatty acids). ④ Containing an abundant vitamin complex, in comparison with another algae, especially vitamin B_{12} and vitamin E. ⑤ Containing pigments such as chlorophyll and carotenoid (β-Carotene) which changes into vitamin A. ⑥ Containing many mineral components.

As to protein, not only quantity but also quality is very important factors to keep animal's lives. The quantity of essential amino acids of Spirulina nearly satisfies the values recommended by the FAO (Food and Agriculture Organization) except for lysine and cystine [3]. Furthermore, the human body needs digestible and absorbable proteins. Fig. 2 shows the comparison of digestibility by pepsin [2]. It is clear from the data shown in Fig. 2 that Spirulina has better digestion properties in comparison to Chlorella.

When human and animals stay in the micro gravity environment for a long time, the phenomena such as calcium decrease in bones and the deterioration of muscular function are reported. Fig. 3 shows the clinical examples for the changes of values of calcium and potassium in blood electrolyte when Spirulina was given every day on the ground [4]. Since these data show the increasing tendencies of calcium and potassium in blood, it is expected some effects to prevent the occurence of these harmful phenomena. From the data described

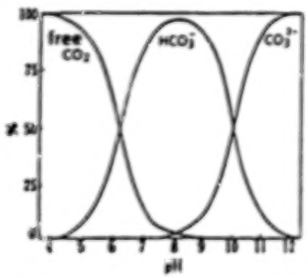


1a. Production of S. platensis by PH - control

$$NaHCO_3 \rightleftharpoons Na^+ + HCO_3^-$$

$$HCO_3^- \rightleftharpoons \frac{1}{2}(CO_2 + CO_3^{2-} + H_2O)$$

$$CO_2 + H_2O \xrightarrow{h\nu} (CH_2O) + O_2$$



1b. The Effect of Existing Conditions of Carbonate in the Medium on PH

Fig. 1. Utilizaiton Efficiency of Carbon Dioxide on Spirulina Cultivation

above, Spirulina seems a good candidate for food in CELSS.

OXYGEN GAS RECOVERING EXPERIMENT

To cultivate algae in the micro gravity environment, many technical problems must be solved. One of those problems is to establish an effective method to gather and recover O_2 gas which is generated by algae and remains in the medium. To separate oxygen gas from the medium, it is not suitable to use a centrifugal separator which would disturb other experimental equipment. Therefore, a membrane separation technique which works without mechanical moving components seems more suitable. As for the membranes, various properties regarding permeability of gas are affected by shapes or material composition of membranes.

Table 1 shows the properties of representative membranes /5/. Picin membrane has a good property for gas permeability, but it has a demerit in mechanical strength if the membrane thickness is reduced in order to get higher permeability. In the result, some compromising between both characteristics is required. Usually O_2 gas generated by algae remains in the form of bubble that is gas itself, in the medium solution. Then, utilization of the porous membrane enabling to pass gas only seems to be better than the plain membrane. Table 1 shows that the membrane made from silicone has a superior property for gas permeation, but, Table 2 /5/ shows that porous type membranes have much better gas permeation characteristics than plain silicone membranes.

For these reasons, we carried on the O_2 gas recovering experiment using a porous hollow fiber membrane module, manufactured by Mitsubishi Rayon Co., Ltd., as the gas separator. The characteristics of this module are shown in Table 3 and the pictures of scanning electron micrographs of hollow fiber are shown in Fig. 4 /6/. One of the hollow fibers is small in inside diameter, and wall thickness. Then, the module which is composed of bundles of hollow fiber is designed very compact. The dimension of this module is 215 mm (L) × 40 mm (D). A very large membrane area can be obtained by using hollow fiber membranes. Fig. 5 shows the block diagram of the O_2 gas recovering experiment equipment now being used in our laboratory. O_2 gas blown into the cultivation tank is carried to the membrane module along the water flow and a part of the O_2 gas permeates the membrane, therefore, for clearing the permeation property of the membrane, the quantity and concentration of permeated O_2 gas are measured by the oxygen gas analyzer.

The cultivation tank is made from acrylic resin and has a round shaped top in order to collect oxygen gas concentratively and also has an inner volume of about 0.009 m^3. For the permeation ability estimation of the membrane, pure water was used and its temperature was set at 30 ℃ (same condition as Spirulina cultivation) and also 99.99% high purity O_2 gas was used insted of the O_2 gas generated by Spirulina.

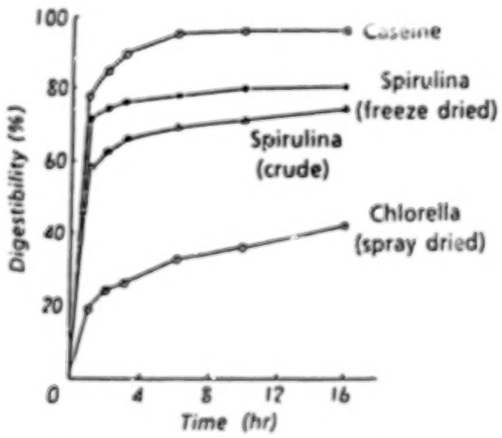


Fig. 2. Digestibility Test by Pepsine

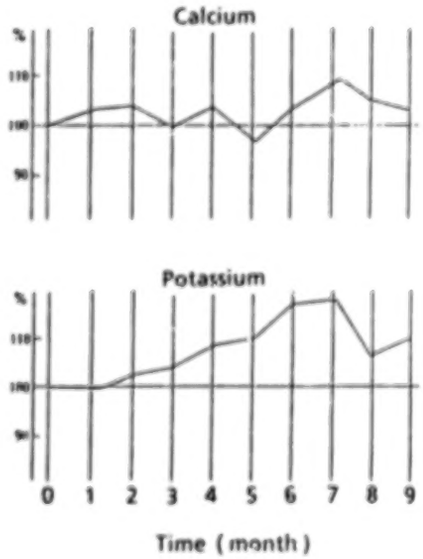


Fig. 3. Variation of Blood Electrolyte

One of the experimental data for O_2 concentration variation of recovered gas is shown in Fig. 6. For determing the accurate dissolved O_2 gas quantity, the dissolved O_2 concentration in pure water was reduced to about 1.7 ~ 1.8 ppm before the experiment. The flow rate of pure O_2 gas from O_2 bottle was 50 cc / min. and the flow time was set for 10 minutes. O_2 gas which permeated the membrane was continuously collected by the suction pump and its concentration was measured by the oxygen gas analyzer. During 4 ~ 5 minutes from the start of the experiment, the concentration of O_2 gas did not change, but after that it rapidly increased. The flow rate of O_2 gas at the membrane module was about 30 ~ 45 cc / min.. Three kinds of membrane area, such as 0.3, 0.5, and 1.0 m^2 were tested, but there appeared only small differences between them.

As shown in Fig. 6, the maximum value of O_2 concentration is about 73%. It took about four hours until the dissolved O_2 concentration went down to the value of the start time. From these results, it is clarified that the membrane module works effectively to recover not only the bubbled but also the disolved O_2 gas.

TABLE 1 O_2 and CO_2 Permeability on Various Polymer

Polymer	O_2 Permeability	CO_2 Permeability
Polyvinyliden Chloride	16	13
Polyester	176	304
Cellulose Acetate	1,705	8,680
Polyethylene	3,500	15,965
	3,200	5,390
	8,800	27,000
Polypropylene	2,900	9,905
Polystyrene	4,850	23,790
Polytetrafluoroethylene	17,600	48,000
Ethyl Cellulose	25,600	104,000
Silicone Elastmer	1,568,000	8,040,000

Membrane thickness 25μ conversion, 25°C, cm^3/m^2·atom·24hr

TABLE 2 Property of Membrane for Artificial Lung

Polymer	Structrue	Total thickness (μ) / Thickness of membrane (μ)	Gas permeability	
			O_2	CO_2
Silicone Elastmer	polyester reinforcement	190/160	140	770
Polysiloxanepolycarbonate	uniformalized	50/50	170	730
Polyalkylsulfon	applied porous polypropylene menbrane	25/2.5	1100	4600
Ethylcelluloseperfluorobutylete	applied polyolefin cloth	175/2.5	880	4700
Polypropylene membrane	porous	25/25	very good	very good
Teflon membrane	porous	500/500	very good	very good

permeability : cm^3 / min. m^2 atom

TABLE 3 Characteristics of Porous Hollow Fiber Module

Material	Polypropylene
Inner diameter	200 μm
Wall thickness	22 μm
Porosity	45 %
Pore Size	0.036 μm
Gas flux	7×10^4 l/m^2 hr 0.5atm
Bubble point	12.5 kg/cm^2
Effective area	0.3, 0.5, 1.0 m^2

GAS CLOSED EXPERIMENT

It was clear that O_2 gas has been obtained from the solution by using the porous hollow fiber membrane module. Then, we have carried out gas closed experiments using the Spirulina cultivator and a fish breeding tank. O_2 gas generated from Spirulina is provided to Tilapia (a kind of tropical fresh water fish) and CO_2 gas exhausted from Tilapia is supplied to Spirulina, through the membrane gas separator and the artificial lung that is the hollow fiber membrane module.

We have shown the block diagram of this experiment system in Fig. 7. Algae cultivator is made from acrylic resin with 180 mm diameter and 300 mm length. The species used in these experiments are Spirulina oscillatoria and Spirulina platensis. The medium solution contained mainly $NaHCO_3$ and its temperature was controlled at 30 °C by thermostat. Light energy was supplied through optical fibers or fluorescent lamp into the cultivator. The time to reach saturation in culture of Spirulina was 7 ~ 10 days in several experiments.

The airtight fish breeding tank is 280 mm diameter × 500 mm length and three Tilapias (length of 15 cm and weight of 160 g) have been bred in it. We plan to use Tilapia as protein supply resource in CELSS and have researched on developing a breeding technology and equipment which works well in micro gravity environment. O_2 gas for Tilapia was supplied through an artificial lung which is the same as the gas seperator and the variation of DO_2 was monitored for this gas closed experiment. The differences of DO_2 values supplied by room air and O_2 from Spirulina cultivator are shown in Fig. 8. The experiment started when the value of DO_2 went down to 0.1 ppm from about 4 ppm. Water circulation rate was 2.0 liter/min. and O_2 gas supply rate was 100 cc/min.. Fig. 8 shows that Spirulina has a good O_2 supply ability. When using Spirulina, the maximum value of O_2 gas concentration reached 46.0 ~ 50.2 %.

CONCLUSIONS

A long term experiment to cultivate Spirulina for more than one month was successfully conducted. Successively, in order to examine the quantity of O_2 gas generated by Spirulina, the closed gas circulation experiment combined the algae cultivator and the breeding tank with three adult Tilapias. The experiment term reached 32 days and it was clean that the continuous experiment operation time depended on the life time of the gas separator.
The ability of the separator was decreased by algae which covered the surface of the membranes. To conduct long term experiments, this problem must be solved, for example, to wash out adhesive Spirulina on the surface of fibers.

Further work is required to establish the technologies to harvest algae automatically, to develop long life gas separators and artificial lungs and to conduct an experiment including not only gas circulation but also food supply to fishes or animals in the complete closed system.

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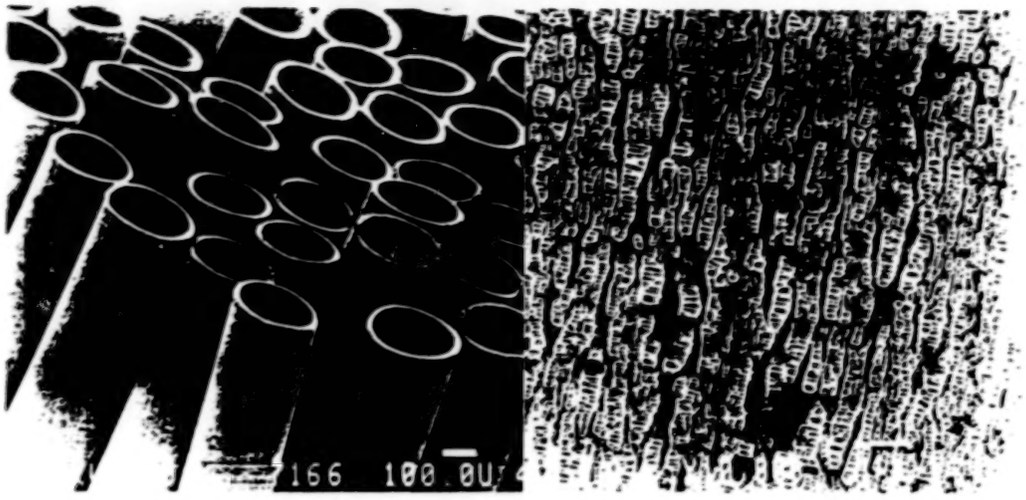

Fig. 4. Picture of Hollow Fiber by Scanning Electron Micrographs

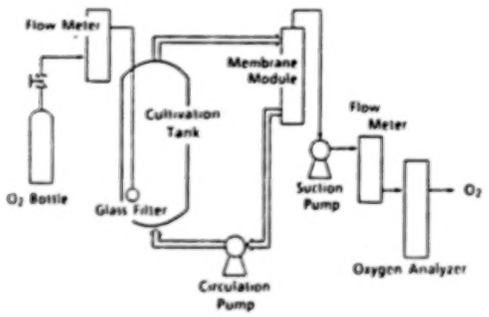

Fig. 5. Block Diagram of O_2 Recovering Experiment System

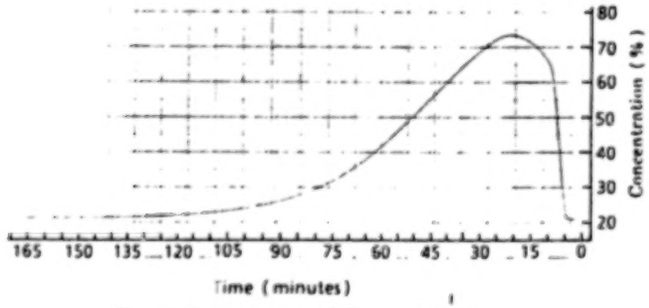

Fig. 6. Concentration of Recovered O_2 Gas

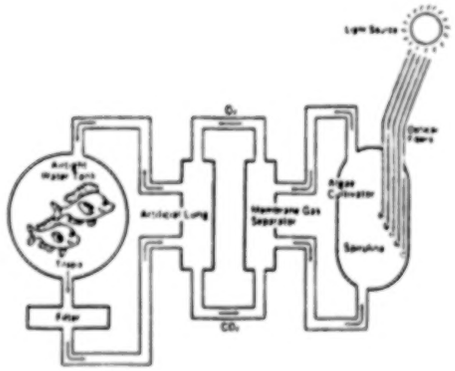

Fig. 7. Block Diagram of Gas Closed Experimental System

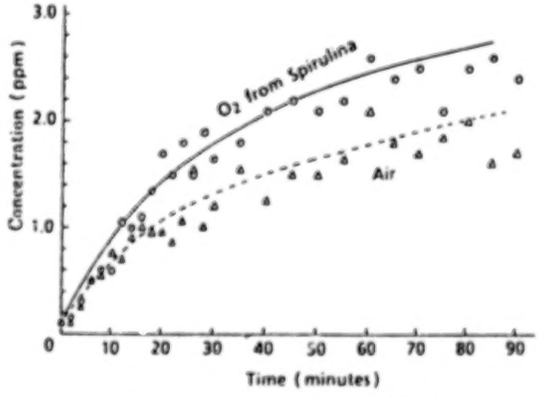

Fig. 8. Concentration of Dissolved Oxygen in Tilapia Breeding Tank

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BIOMASS RECYCLE AS A MEANS TO IMPROVE THE ENERGY EFFICIENCY OF CELSS ALGAL CULTURE SYSTEMS

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ABSTRACT

Algal cultures can be very rapid and efficient means to generate biomass and regenerate the atmosphere for closed environmental life support systems. However, as in the case of most higher plants, a significant fraction of the biomass produced by most algae cannot be directly converted to a useful food product by standard food technology procedures. This waste biomass will serve as an energy drain on the overall system unless it can be efficiently recycled without a significant loss of its energy content.

We report experiments in which cultures of the alga Scenedesmus obliquus were grown in the light and at the expense of an added carbon source, which either replaced or supplemented the actinic light. As part of these experiments we tested hydrolyzed waste biomass from these same algae to determine whether the algae themselves could be made part of the biological recycling process. Results indicate that hydrolyzed algal (and plant) biomass can serve as carbon and energy sources for the growth of these algae, suggesting that the efficiency of the closed system could be significantly improved using this recycling process.

INTRODUCTION

The operation and utility of a CELSS, a biologically driven and maintained life-support system that can be considered as a small biosphere, will be subject to the same rules and limitations as any other photosynthetically driven system. This is particularly true of energy flow. Since energy will undoubtedly be one of the limiting commodities in any long-term space-flight mission, the use and reuse of energy during the course of the mission will be of fundamental importance.

The efficiency of energy utilization in photosynthetically-driven systems -- be they algae-based, higher-plant-based, or as is most likely, a combination of the two -- has two fundamental limitations with respect to energy utilization and conservation:

1) Photosynthetic systems can harvest at most 20% of the (white) light energy that is absorbed by the photosynthetic tissue. (This value can be increased to ca. 30% if relative monochromatic red light can be efficiently supplied, although this may have deleterious secondary consequences in the case of many higher plants.)/1/

2) Only a relatively small fraction of the plant or algal product will generally be useful as food for the crew. In most cases only part of the plant or alga will be consumed by the crew, the remainder being discarded during the course of preparation.

The losses occuring due to the considerations of item #1 may be immutable (at least in the relatively near term) and thus will not be considered further in this communication. Instead, we will concentrate our efforts on item #2, and explore means by which this energy drain could be alleviated or eliminated.

The yield potential of agronomic crops has been substantially increased through breeding programs whose goal was the development of plants that provide a greater percentage of useful biomass (e.g. seeds, edible stems, tubers, etc.) to total biomass. This proportion, called the harvest index, is a measure of how the products of photosysthesis (and indirectly light energy) are partitioned. Table 1 is a compilation of the harvest index of a number of crop plants, some of which are currently being considered for a CELSS. Note that this value is generally below 50%, indicating that for most plants more than one-half of the energy photosynthetically fixed would be unavailable to the CELSS biosystem. A similar, though less clear-cut case can be made for the case of algae, particularly Scenedesmus, Chlorella and Spiruline (Table 2). In this instance roughly 50% of the biomass is in the form of protein that is beneficial to humans. If these algae were to be produced and harvested only for their protein content, the remainder would be waste biomass.

These data suggest that, without special provisions, about one-half of the captured energy of the food plants of a CELSS would be degraded via the waste-handling system. One possible means for surmounting this problem would be to recycle this waste biomass; i.e., use the energy content and associated carbon skeleton structures of the waste biomass to produce new useful biomass. In this way, one would be able to minimize the intervening oxidative (waste-processing) steps, which basically degrade high-grade light energy to low-grade heat energy without the extraction of useful metabolic energy. In essence, we would attempt to extract useful "work" from otherwise

unusable biomass.

TABLE 1. Harvest index of some crop species. The harvest index in the percentage of total aerial dry weight at maturity that represents economic yield (grain or seed). Adapted from ref. 2.

Crop	Harvest Index (%)	
	Average	Range (different species)
Maize, hybrids	42	38 to 47
Sorghum	41	40 to 41
Rice	51	43 to 57
Barley	48	35 to 52
Wheat	35	23 to 46
Rye	27	27 to 29
Dry bean	59	53 to 67
Soybean	32	29 to 36

TABLE 2. Chemical Composition of Different Algae (% dry matter) Adapted from ref 3.

Component	Scenedesmus	Spirulina	Chlorella
Crude Protein	50 to 55	55 to 65	40 to 55
Lipids	8 to 12	2 to 6	10 to 15
Carbohydrates	10 to 15	10 to 15	10 to 15
Crude fiber	5 to 12	1 to 4	5 to 10
Ash	8 to 12	5 to 12	5 to 10
Moisture	5 to 10	5 to 10	5 to 10

There are several options available for the heterotrophic component this recycle system: yeast, which have a long history of useful controlled fermentative growth, some bacteria, and some algae. [We should note that higher (crop) plants cannot function in this role.] In this communication we will concentrate on the use of algae.

Under normal conditions, most algae grow photosynthetically, but some strains have the ability to use alternate growth modes (see e.g. ref 4). This photosynthetic growth mode, in which cell carbon is obtained from the carbon dioxide (CO_2) in the gas phase and metabolic energy is obtained from sunlight, is often referred to as photoautotrophy. At the opposite extreme, so-called chemoheterotrophy, cell carbon and metabolic energy are both derived from organic compounds (nutrients). Other, middle-ground, growth modes also are observed in some algae. In the case of photoheterotrophy, growth is maintained at the expense of organic compounds which are taken up, or photoassimilated, at the expense of light energy. In another mode of growth, so-called mixotrophy, there is a simultaneous assimilation of organic carbon sources and CO_2 in the light in amounts that vary with culture conditions.

In the present communication we will describe some experiments in which we attempt to use some of these alternate modes of algal growth to produce the green alga Scenedesmus obliquus. Our results suggest that this organism can be produced in this manner, and that these alternate modes of growth could provide the means to significantly increase the overall energy utilization efficiency of a CELSS.

MATERIALS AND METHODS

Culture Conditions and Sampling Techniques.

Growth of S.obliquus was achieved in a mineral salts medium, supplemented where appropriate with heterotrophic carbon sources. The Basal Medium contained per liter: KNO_3, 2.0g; K_2HPO_4, 0.19g; KH_2PO_4, 0.075g; $MgSO_4.7H_2O$, 0.5g; $CaCl_2.2H_2O$, 0.01g; Hutner's Mineral Salts /57, 1.0 ml. The carbon source for this medium was provided by bubbling with 2% CO_2 in air. If required, solid medium for growth of S.obliquus was prepared by addition of glucose (0.5%, w/v) and Bacto agar (1%, w/v) to the above medium.

Small scale cultures were maintained in 15 ml capped test tubes containing 3 ml of Basal Medium plus glucose and incubated on a rotary drum at 28°C and low light (40μE m^{-2} sec^{-1}). Larger scale cultures, for growth experiments, were grown in Roux bottles containing 700 ml of Basal Medium. Mixing was achieved by bubbling with CO_2 or air. The culture bottles were enclosed in a blackened box that had only one side open to the light. This served two purposes: (i) it minimized the amount of reflected light reaching the culture, and (ii) it allowed the level of incident light to be varied by use of cheesecloth screens over the open side of the box.

To facilitate intermittent sampling, the Roux bottles were sealed with a rubber stopper pierced by two stainless steel sparging needles and a vent opening. A sterile 20 ml syringe was attached to one of the needles and CO_2/air was admitted to the culture via the other. To remove a sample, the vent was clamped shut, whereupon the mounting air pressure inside the vessel forced algal suspension into the removable syringe.

Preparation of Algal Extracts

A water soluble extract of algal biomass was prepared in the following way. Dried algal cells (20 g) were homogenized thoroughly in 200 ml of chloroform/methanol (1:1, v/v) and extracted for 24 h to remove lipid and pigments. The organic extraction was repeated twice. The extracted biomass was collected by centrifugation, dried and resuspended in 100 ml of water. Cellulase was added to a final concentration of 0.1 mg/ml and the mixture was incubated at 25°C, with stirring, for 24 h. Particulate material was removed by centrifugation and the remaining water soluble extract was heated in a boiling water bath for 20 min to inactivate the cellulase. This heating step also reduced the volume of extract to approximately 50 ml. The extract was sterilized by filtration through 0.2µm filters prior to use.

No attempt was made at this stage to quantitate the level of organic material, or specifically glucose, in the extract. Where appropriate, the extract was included in the growth medium at a level of 2%, v/v.

RESULTS AND DISCUSSION

Before we could ascertain whether organic compounds had any effect on autotrophically grown S.obliquus, it was necessary to determine culture conditions in which light was limiting growth. Therefore, algal cultures were grown autotrophically at different incident light intensities in order to determine a truly growth-limiting light intensity. We observed that above an incident light intensity of about 70µE m^{-2} s^{-1} the growth rate of S.obliquus was independent of light intensity in our system, i.e., the light was saturating (data not shown). Figure 1 shows the autotrophic growth kinetics observed at three light intensities, namely 46, 80 and 144 µE m^{-2} s^{-1}. Note that an incident light intensity of approximately 46 µE m^{-2} s^{-1} resulted in a growth rate equivalent to half the maximum observed rate. Accordingly, subsequent experiments requiring light-limited growth of S.obliquus were performed at an incident light intensity of approximately 50µE m^{-2} s^{-1}.

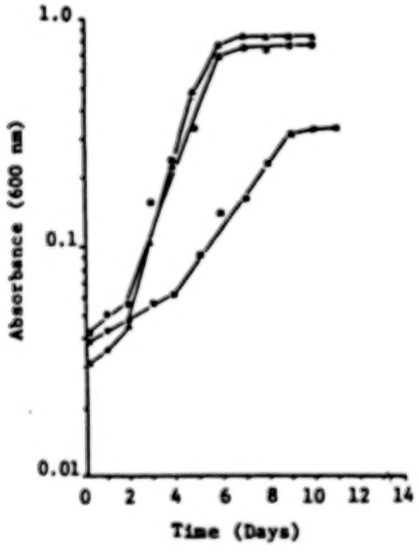

Fig. 1. Culture dry weight as a function of time for growth of S.obliquus at different light intensities. Cultures were grown in Roux bottles containing basal medium, bubbled with 2% CO_2. Cell growth was assayed by performing dry weight measurements on duplicate 20 ml samples taken at daily intervals. Light intensities in µE m^{-2} s^{-1}: o---o,80 ; □---□,46 ; Δ---Δ,144

Table 3 summarizes the results of a series of experiments in which we tested the ability of various carbon sources to support or supplement the growth of S.obliquus. In these experiments the organisms were grown either in the dark or under limiting light, and the growth medium supplemented with various carbon sources; i.e., carbon dioxide (either 0.03%, the amount in air, or air amended with 2% CO_2), glucose (0.5%, w/v), or algal extract (2%, v/v). For each growth condition cell number was monitored as a function of time. These data were graphed, and the minimum doubling time for each culture was determined from the graph. The maximum growth rate (µ) was then calculated from the doubling times. Each value in Table 3 represents the mean of two separate experiments.

TABLE 3. Growth rate and doubling time of S. obliquus as a function of available energy source.

GROWTH CONDITION	CARBON SOURCE	DOUBLING TIME (h)	GROWTH RATE (μ)
1a) light/autotrophic	air (CO_2)	50.7	0.014
b) light/autotrophic	2% CO_2	28.8	0.024
c) dark/autotrophic	2% CO_2	n.d.	0
2a) light/mixotrophic	glu + air (CO_2)	16.1	0.043
b) light/mixotrophic	glu + 2% CO_2	15.6	0.044
c) heterotrophic (dark)	glu + 2%CO_2	18.9	0.037
3a) light/mixotrophic	extract + air (CO_2)	23.5	0.029
b) light/mixotrophic	extract + 2% CO_2	23.0	0.030
c) heterotrophic (dark)	extract + 2% CO_2	24.2	0.029

The first two items in Table 3 (items 1a & 1b) show the growth rates and doubling times observed when S. obliquus was cultured under limiting light (ca. 25 $\mu E\ m^{-2}\ s^{-1}$) with unamended air (containing 0.03% CO_2) and air amended with 2% CO_2. Note that even under these limiting light conditions the growth rate was increased substantially by increasing the CO_2. We should also note that the maximum rate observed in these experiments (μ=0.024) is about a factor of four less than the maximum rate observed with these organisms in our hands under saturating light and CO_2. Item 1c is the "dark control".

Items 2a-2c show the effect of added glucose on the growth rates. Under these low-light conditions the growth rate of S. obliquus in the presence of added glucose was almost double the rate observed in autotrophic cultures at the same light intensity. Note that there was very little effect of added CO_2; the observed rates at 0.03% CO_2 (air) and 2% CO_2 were almost identical. This observation is confirmed by the results shown in item 1c: the glucose-supported growth rate in the absence of light was substantially higher than the autotrophic rate under the same light intensity, and was within ca. 20% of the mixotrophic rate. Thus under these conditions the glucose-supported heterotrophic rate is higher than the CO_2-supported autotrophic rate and is within a factor of two to three of the maximum observed rate under saturating light and CO_2.

As shown in items 3a-3c, algal extract also supported a higher rate than that observed in autotrophic cultures, though the observed effect was less than with glucose. Again, as in items 3a and 3b above, there was no appreciable difference between the rates observed with or without added CO_2, and indeed, little significant effect of light at all. The observed lack of additivity of the dark and light rates may reflect the existence of a "Kok effect" i.e., the suppression of respiration by photosynthesis /6/. Whatever the ultimate cause, these results do indicate that an algal extract prepared from S. obliquus can support the growth, either heterotrophically or mixotrophically, of this same organism.

CONCLUSIONS

Our results show that hydrolyzed S. obliquus biomass can serve as a carbon and energy source for the growth of these same algae. This finding suggests that the efficiency of biomass production in a CELSS might be significantly increased by recycling unusable waste biomass, generated from the production of food from algal and plant biomass, through the algal system for the production of additional whole algal material.*

We wish to emphasize that a scheme of this type may provide the means to avoid, or at least minimize, futile energy cycles associated with carbon flow in a CELSS. The only significant energy input into the biological carbon cycle is through photosynthesis-related processes, which use this energy to produce the reduced carbon compounds that comprise higher plant and algae. This energy is subsequently either harvested (via biological oxidation processing by the crew) or lost via spurious oxidation or waste processing. As shown in Figure 2, the most straightforward, but energy inefficient pathway of carbon flow would be a scheme in which plant and algal waste materials would be recycled via the waste processing system. A more complex, but more energy-efficient pathway would involve a scheme in which this route were short-circuited, and the reduced, and energy-rich waste biomass reused before oxidation. Such a recycling system could result in increases in the energy efficiency of carbon flow (and air regeneration) approaching 50%, compared to a system in which this non-edible material is oxidatively decomposed by standard waste handling techniques.

*An important point, not addressed by the rather preliminary experiments discussed above, is the relative efficacy of the different algal fractions for the support of algal growth. Ideally, the various algal fractions that cannot be converted to a useful component of the human diet would, after proper processing, prove to be efficient carbon and energy sources for Scenedesmus. However, at present we cannot rule out the possibility that the fractions supporting algal growth in the above experiments are the very same fractions that are best suited for the production of human food. A definitive answer to this question would require a more detailed study of both the food processing technologies applicable to Scenedesmus and detailed heterotrophic and mixotrophic growth studies of this alga.

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N88-12255

OPERATION OF AN EXPERIMENTAL ALGAL GAS EXCHANGER FOR USE IN A CELSS

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ABSTRACT

Concepts of a CELSS anticipate the use of photosynthetic organisms (higher plants and algae) for air revitalization. The rates of production and uptake of carbon dioxide and oxygen between the crew and the photosynthetic organisms are mismatched. An aglal system used for gas exchange only will have the difficulty of an accumulation or depletion of these gases beyond physiologically tolerable limits (in a materially closed system the mismatch between assimilatory quotient (AQ) and respiratory quotient (RQ) will be balanced by the operation of the waste processor). We report the results of a study designed to test the feasibility of using environmental manipulations to maintain physiologically appropriate atmospheres for algae (*Chlorella pyrenoidosa*) and mice (*Mus musculus* strain DW/J) in a gas-closed system. Specifically, we consider the atmosphere behavior of this system with *Chlorella* grown on nitrate or urea and at different light intensities and optical densities. Manipulation of both the photosynthetic rate and AQ of the alga has been found to reduce the mismatch of gas requirements and allow operation of the system in a gas-stable manner. Operation of such a system in a CELSS may be useful for reduction of buffer sizes, as a backup system for higher plant air revitalization and to supply extra oxygen to the waste processor or during crew changes. In addition, mass balance for components of the system (mouse, algae and a waste processor) are presented.

INTRODUCTION

A Controlled Ecological Life Support System (CELSS) is one option for maintaining human life during extended space flight. A CELSS uses energy to recycle matter through an integrated variety of biological and physical processes, thereby regenerating consumable supplies. The objective of the NASA-sponsored CELSS program is to investigate the feasibility of producing food and revitalizing atmospheres by growing algae, higher plants, and processing wastes by microbial or physical-chemical oxidation /1/.

Revitalizing the atmosphere within a spacecraft or planetary habitat requires that the crew be continuously supplied with oxygen (O_2) and that carbon dioxide (CO_2) be removed from the system. Physico-chemical methods are available to remove and reduce CO_2 and generate O_2 /2/. The presence of algae and higher plants in a CELSS allows the photosynthetic uptake of CO_2 and production of O_2. Therefore, physico-chemical methods of air revitalization are not envisioned as part of a CELSS except, possibly, as a backup system.

The use of biological processes to maintain physiologically appropriate concentrations of CO_2 and O_2 requires the development of strategies which maximize system reliability and stability while using the inherent characteristics of the organisms as control points. Previously we have reported on the use of environmental manipulations to stabilize the behavior of a gas-closed mouse-algal system /2/.

We report here the recent results obtained from the operation of this system (Figure 1). Specifically, we consider the atmospheric behavior of the gas-closed system using cultures of *Chlorella pyrenoidosa* and a dwarf mouse

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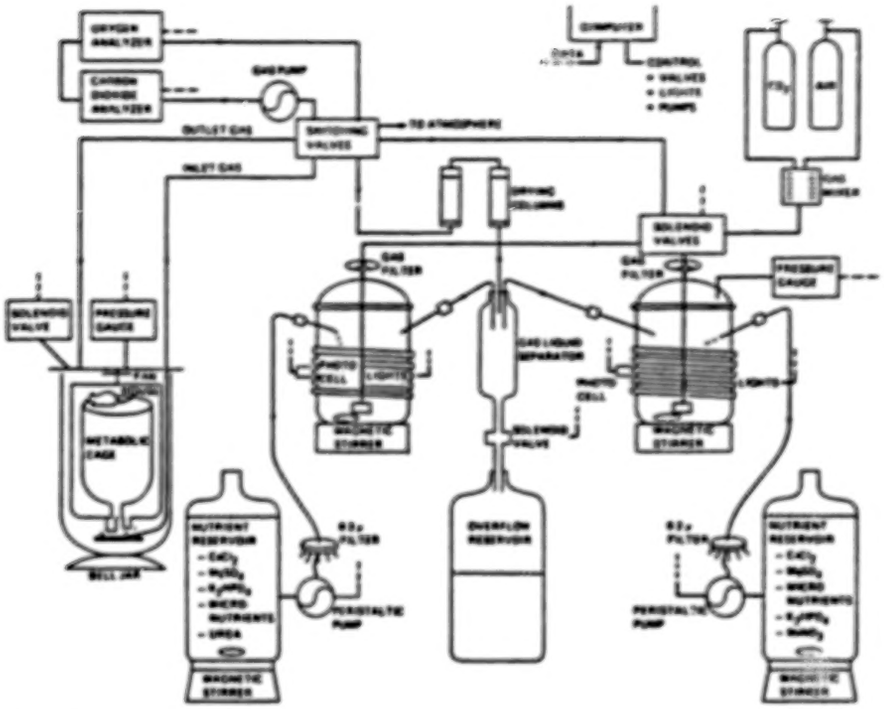

Fig. 1. Experimental mouse-algal system.

Mus musculus dw/j. Additionally the results of preliminary mass balance studies of the system are presented, including the use of an abiotic waste processor.

MATERIALS AND METHODS

A variety of photosynthetic gas exchange experiments were conducted using the system (Figure 1). Gas analyzers measured changes in partial pressures of CO_2 and O_2 within the system. The system was operated with algae only, a mouse only, and with the algal and mouse reactors coupled.

Measurement of algal assimilatory quotients (AQ = moles CO_2 consumed/moles O_2 produced) were made by closing the algal reactor to the ambient atmosphere. The algal cultures are normally supplied with CO_2 (2%) enriched air. When AQ measurements were made, the gas flow from the cylinders was stopped and the gas within the system was recirculated using a pump. The slopes of the CO_2 and O_2 concentrations were used to calculate the AQ.

Measurement of mouse respiratory quotients (RQ = moles CO_2 produced/moles O_2 consumed) were accomplished by closing the mouse in the chamber with ambient atmospheric concentrations of CO_2 and O_2 and observing the changes in each gas concentration over time. The RQ was calculated from the change in CO_2 divided by the change in O_2. Respiratory quotients were measured for short time periods (one or two hours) which included awake, sleeping and eating periods.

Combined algal-mouse experiments were conducted with the algal reactor and the mouse chamber linked and closed to the exchange of gas with the ambient atmosphere. Two algal vessels were required to supply the O_2 requirements of the dwarf mouse. Algal growth conditions (light intensity and cell density) were varied to control the photosynthetic *rates* of each culture. Different nitrogen species (urea and nitrate) were used in the algal growth medium to change the *ratio* of CO_2 uptake to O_2 release. Combinations of both rate and ratio manipulations were used to determine the optimal operating conditions (*i.e.* nitrogen source, light intensity, optical density) for maintenance of atmospheric levels of CO_2 and O_2 within the physiological tolerances of both the algae and the mouse.

Bacterial populations within the algal cultures were monitored by plating samples onto trypticase soy agar (TSA) plates, incubated for 48 hours at 30 C. Plate counts for both bacteria and algae were made using the spread plate method and TSA plates.

Carbon, hydrogen and nitrogen percentage (by weight) of the algae, mouse food and feces were determined by running lyophilized samples on a Perkin-Elmer model 240B elemental analyzer. To determine percent oxygen, ash percent was determined and oxygen calculated by difference. The contribution of other elements (sulfur, phosphorus, etc.) was assumed to be negligible.

Mass balance determinations for the mouse were made by measuring the inputs to (food and water) and outputs from (feces, urine, unconsumed food and water) sets of three mice housed in a metabolic cage not connected to the gas exchange system. Elemental analysis of these materials was conducted and averaged for a series of 15 runs. Assumptions were made concerning the magnitude of exhaled water from the mice and values for percentages of water in feces and urine were obtained from the literature /4/. Mass balance of the wet-air oxidation of feces was determined from a set of runs conducted in a system physically distinct from the gas exchange system.

RESULTS AND DISCUSSION

Atmosphere Revitalization

Metabolic differences between the mouse and algae result in an inherent mismatch between AQ and RQ leading to depletion of either CO_2 or O_2, depending upon the direction and degree of the mismatch /3/. This mismatch is more evident in a materially-open system while in a materially closed system, the operation of a waste processor will reduce the impact of the AQ-RQ mismatch. Therefore, in order to maintain stable concentrations of CO_2 and O_2 within a coupled algal-mouse system, it is necessary to more closely control the AQ and the RQ. Control of the RQ is possible but not a realistic option for use in a CELSS because it would require excessive restrictions on crew diet and activity. Thus, techniques to match the AQ of the algae to the RQ of the mouse have been examined. One factor that influences AQ is the observed difference between nitrate and urea grown cultures /5/. The average AQ of axenic cultures of *Chlorella pyrenoidosa* grown on nitrate was 0.67 ± 0.02 (n=5) and on urea was 0.80 ± 0.04 (n=3) over a range of light intensities and optical densities.

The AQ of cultures grown on nitrate and urea differ because nitrate is a more oxidized form of nitrogen than urea. As urea is metabolized to ammonia, CO_2 is released, which raises the AQ with respect to nitrate. This can be shown by considering the composition of *Chlorella pyrenoidosa*, as determined by elemental analysis, $C_{5.77}$: $H_{9.30}$: $O_{2.47}$: $N_{1.00}$. The incorporation of CO_2, water and either nitrate or urea into *Chlorella pyrenoidosa* and O_2 can be expressed for nitrate:

$$5.77CO_2 + 4.65H_2O + NO_3^- = C_{5.77}H_{9.30}O_{2.47}N + 8.36O_2 \quad (1)$$

yielding a theoretical AQ of 5.77/8.36 = 0.69.

for urea:

$$5.27CO_2 + 3.65H_2O + 0.5(N_2H_4CO) = C_{5.77}H_{9.30}O_{2.47}N + 6.11O_2 \quad (2)$$

yielding a theoretical AQ of 5.27/6.11 = 0.86. This discussion demonstrates how the differing oxidation states of nitrate and urea affect the AQ. The theoretical values are close to the experimental values.

The presence of bacteria in the algal cultures was found to affect CO_2 and O_2 levels within the system. The bacteria present were contaminants and were not intended to represent the decomposer (waste processing) functions of a CELSS. The contamination could have occurred by several routes, specifically by failure of the media input filters, back-contamination from the mouse chamber or during sampling procedures. Bacterially contaminated cultures exhibited a decrease in the apparent algal AQ and an increase in the variation of AQ. Contaminated nitrate cultures grown under a range of light intensities and optical densities had an average AQ of 0.50 ± 0.07 (n=25) while uncontaminated nitrate cultures had an AQ of 0.67 ± 0.02 (n=5). Contaminated urea grown cultures had an AQ of 0.77 ± 0.12 (n=28) while the uncontaminated urea cultures had an AQ of 0.80 ± 0.04 (n=3). This decrease is due to bacterial respiration consuming O_2 and producing CO_2 at a different ratio than algal photosynthesis was producing O_2 and consuming CO_2 (*i.e.* bacterial RQ ≠ algal AQ). In an operational CELSS, in which bacterial contamination will occur, reduction in the apparent AQ must be compensated for by an increase in the photosynthetic rate of the algal cultures, or finding operating modes that yield AQ's greater than the crew RQ. Control of respiratory gas concentrations will be most effective if the algal systems bracket the crew RQ.

Bacterially contaminated cultures grown on nitrate exhibit a decrease in apparent AQ and a large AQ variation. The large variation in AQ is likely due to different bacterial populations as well as to population sizes. We concluded that even low levels of bacterial contamination affect the apparent AQ of the algal culture. To determine how a mixed bacterial-algal microbial community affects the apparent AQ, further data must be collected regarding the bacterial community diversity and relative population sizes of bacteria and algae within the system.

In a CELSS, the composition of mixed algal-bacterial cultures will need to be monitored in order to maintain defined communities which effectively regenerate atmospheres. Our experience indicates that it is difficult to maintain axenic algal cultures for long time periods (weeks) and to attempt this in a space-based system will be both impractical and unnecessary for long-duration (years) space missions. Large fermentors would be very difficult to sterilize and the equipment required would consume more power, volume and maintenance time and

have too large a launch mass to be practical. The cost of maintaining axenic cultures would be much greater than the advantage gained.

The RQ of the dwarf mouse was 0.97 ±0.06 (n=5), this agrees with values up to 0.97 for dwarf mice reported by Eley and Myers /6/. The mean RQ for humans is 0.82 /7/. Variation of diet and/or activity levels results in different RQ values. The dietary ratio of carbohydrate, lipid and protein are known to affect RQ because of different oxygen requirements for the oxidative metabolism of foods. Activity levels affect RQ because the nature of oxygen utilization varies with the metabolic requirements of the animal. Since the air revitalization system must be responsive to normal variation in crew diet and activity levels there were no restrictions on the diurnal activity or diet of the mouse. Since the mouse RQ is higher than the human RQ, changes in the algal operating conditions will need to be explored for human-scale experiments.

A CELSS air revitalization system will be designed to mininize the RQ-AQ mismatch. However, because the range of AQ control is limited, control will also depend on the ability to manipulate rates of photosynthesis. In combined algal-mouse experiments, variation of light intensity controls gas exchange mismatches by affecting photosynthetic rates through culture density. Decreasing or increasing incident light intensity directly affects the concentrations of CO_2 and O_2 within the system atmosphere by changing the photosynthetic rate of the culture.

Figure 2 illustrates the crossover area (where photosynthesis ~ respiration) between photosynthetic mode (*i.e.* oxygen production of the algae exceeds the oxygen uptake of the mouse) and respiratory mode (*i.e.* oxygen consumption of the mouse exceeds oxygen production by the algae). The crossover area curves exhibit the difference in algal AQ between nitrate (Figure 2a) and urea (Figure 2b) grown cultures. Predictions can be made with this data regarding the overall system state for cultures maintained at selected optical densities, light intensities, and nitrogen sources. Consequently, it is possible that multiple reactors, each at selected operating regimes and with distinctive gas exchange characteristics, may be used to provide flexibility for control of the system.

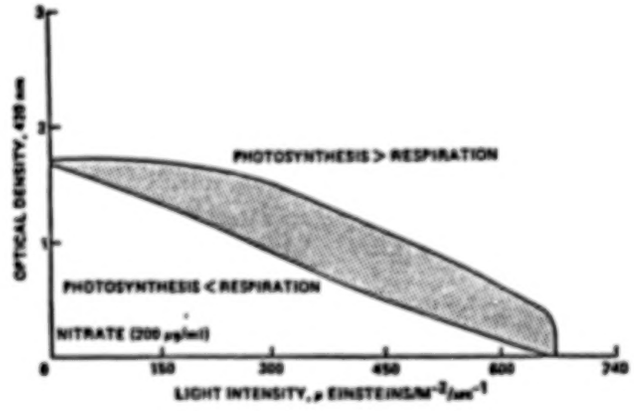

Fig. 2a. Crossover area as a function of optical density and light intensity.

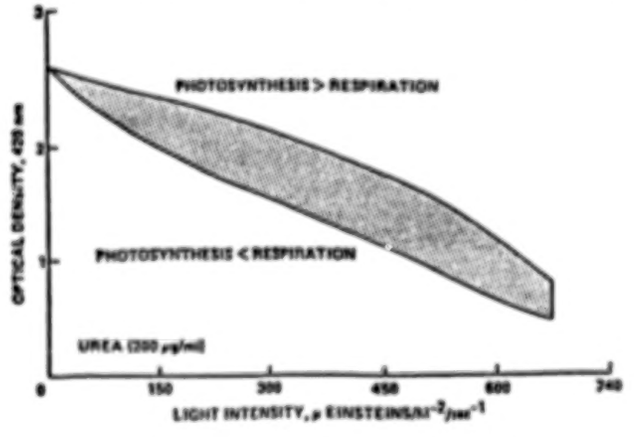

Fig. 2b. Crossover area as a function of optical density and light intensity.

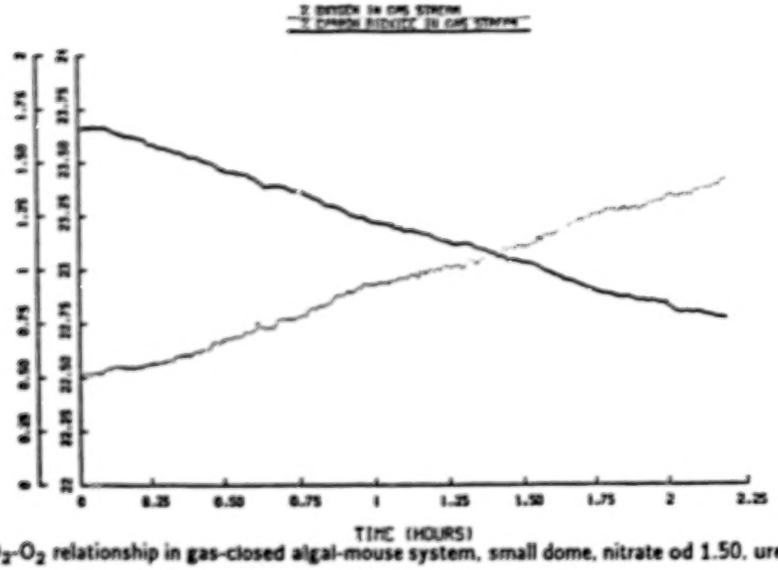

Fig. 3. CO_2-O_2 relationship in gas-closed algal-mouse system, small dome, nitrate od 1.50, urea od 4.50.

Combined algal-mouse runs were conducted using both the large and small mouse chambers coupled to the two algal reactors, one growing on nitrate and the other on urea. Figure 3, a small mouse chamber run, shows the concentration of O_2 increasing with a corresponding decrease in the CO_2 concentration. This photosynthetic mode can be explained by considering the operating conditions of each algal reactor. The high optical densities of the two cultures led to an overall system state in photosynthetic excess as predicted in Figure 2.

A large mouse chamber run (Figure 4) exhibits a system state in which the concentration of O_2 is decreasing with a corresponding increase in the CO_2 concentration. Similarly, the reactor conditions of Figure 4, as seen in Figure 2, would lead to a system state in respiratory excess which is demonstrated experimentally. As can be seen in Figure 3, the timescale for significant changes to occur in the gas composition of the small chamber is between 1 and 2 hours. Since the timescale will scale as the system volume, significant changes in the large chamber require a timescale of roughly 18 to 36 hours. Thus, Figures 3 and 4 demonstrate that system states can be predicted from the crossover area curves and that these predictions are independent of the buffer volume of the mouse chamber.

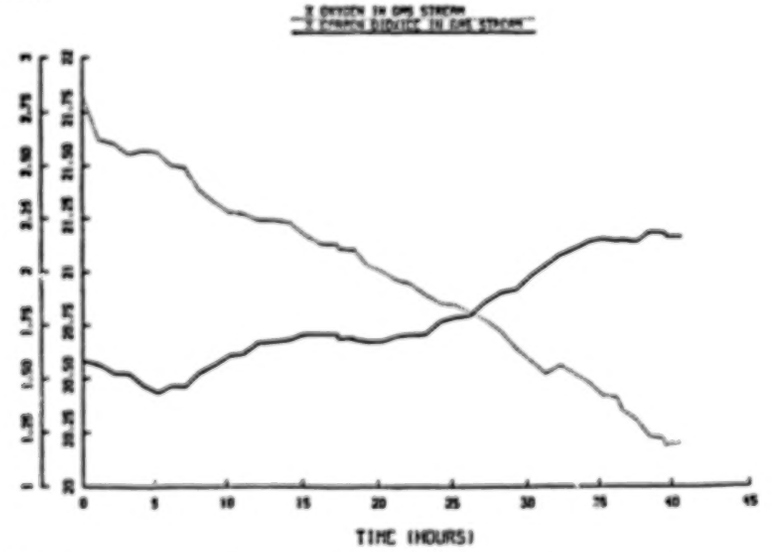

Fig. 4. CO_2-O_2 relationship in gas-closed algal-mouse system, large dome, nitrate od 0.95, urea od 1.45.

The observed buffering capacity of the large chamber might appear useful as a control point. However, responsiveness of the system decreased with the larger system volume which effectively increased the time required to recover from perturbations. Furthermore, minimization of reservoir sizes reduces the weight, volume and hence cost of a CELSS and consequently large buffers are not practical.

Figure 5, which illustrates the system operating in a stable fashion over 2-3 system timescales, demonstrates that long-term stability is possible by combinations of rate and ratio manipulations. The initial photosynthetic mode was mitigated by lowering the optical density of the urea reactor at 3.0 hours. At about 10.0 hours the decreasing CO_2 and increasing O_2 slopes showed a decrease in magnitude. However, a further optical density decrease of both reactors was necessary to change the slopes to a value close to zero. Maintenance of these conditions allowed the system to remain in a stable state.

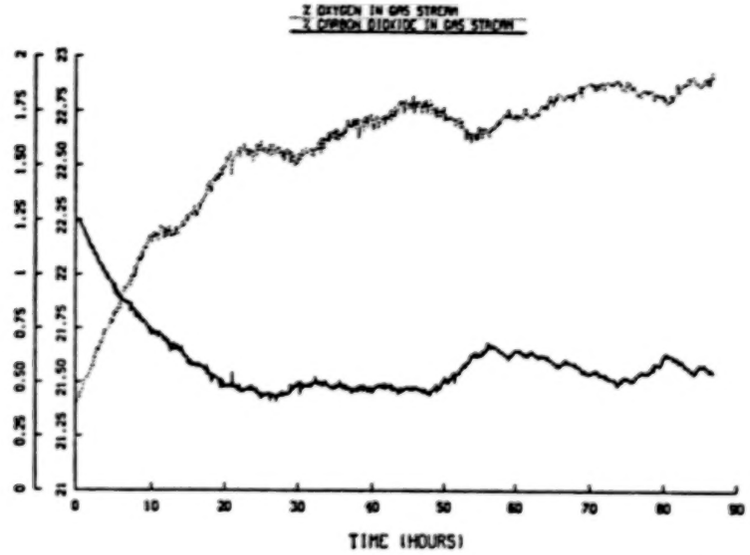

Fig. 5. CO_2-O_2 relationship in gas-closed algal-mouse system, large dome, nitrate od 0.80, urea od 0.90.

The initial photosynthetic mode observed is contrary to crossover area curves predictions (Figure 2). Variation of bacterial community diversity and population size will alter the photosynthetic capability of the system and lead to disparity between predictions and observations. Additionally, differences in RQ's between mice may account for part of the variability. Further, combined nitrate, urea system behavior may not be the same as separate nitrate or urea system behavior from which the crossover area curves of Figure 2 were derived.

We conclude that environmental manipulations of algal growth conditions can significantly reduce the inherent mismatches in respiratory gas requirements of a photoautotroph-heterotroph system. The use of environmental controls has the advantage of limiting the amount of intensive control or external devices required to maintain stability. Our data indicate that photosynthetic rate is easily manipulated by variation of light intensity and optical density. AQ may be manipulated by choice of nitrogen source. The effects of bacterial contamination can be minimized by aseptic culture techniques and monitoring of population size and diversity. The ultimate goal will be to maintain well defined algal-bacterial communities. The effects of buffer size must also be taken into account in the design and operation of an algal gas exchanger in order to allow realistic timescales for system control and to minimize cost.

Further experimental work must be completed to determine the precise operating conditions required to maintain atmospheric stability within a closed system. Once such equilibrium conditions are established, an algal gas exchanger will be able to operate reliably, with minimal external manipulations, and be able to respond appropriately to the respiratory gas requirements of a human crew. Given a scaled version of the algal system described, with the same surface to volume ratio and equivalent light intensity, we calculate that 300 liters of algae would be required per person per day. Improvements in light use efficiency and reactor design will decrease the volume of culture required to support a human crew.

Mass Balance

The maintenance of concentrations of CO_2 and O_2 within acceptable physiologic ranges indicates that endogenous control of the photoautotrophic (algal) component allows atmospheric stability to be sustained. To determine the degree of closure through the system, quantification of elemental flows through system compartments is required. In addition to the respiratory gases, carbon and oxygen cycle through living tissue, feces, urine, water, mouse food and algal nutrients. The rates and concentrations are measured in order to more accurately assess the stability of the system.

The system elemental flows (carbon, oxygen, nitrogen, hydrogen (C,O,N,H)) are quantified as they pass between three compartments: mouse, algal and waste processor (Figure 6). Elemental analysis of mouse food, feces,

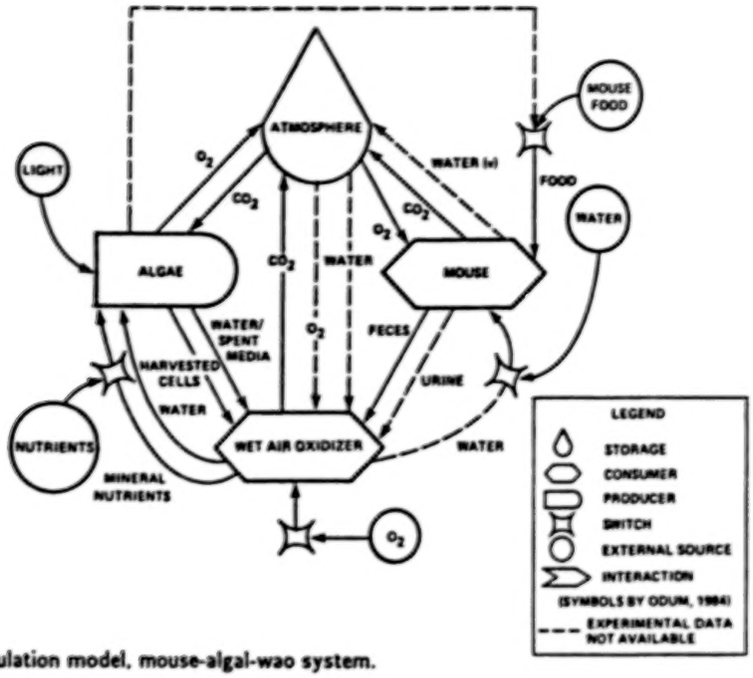

Fig. 6. Simulation model, mouse-algal-wao system.

urine and algal cells and the known elemental composition of algal media, waste processor inputs and measured waste processor outputs are used to calculate mass balances through and between system compartments.

Several assumptions are made within this approach. To achieve system closure the algal cells must be used as mouse food, although this has not been done experimentally. Given the known composition of the mouse food and algal cells, it would be possible to theoretically "replace" the mouse food with algal biomass. However, the nutritional quality and the response of mouse metabolism to an algal diet is not well defined. The composition of spent algal media is not directly measured, but it may be calculated from the known initial composition minus the measured algal cell uptake values. The exhaled water vapor from the mouse is not directly measured so it was calculated by balancing oxygen output. These assumptions allow a preliminary analysis of system mass closure but a more thorough analysis must await refinement of system capabilities.

To analyze mass flow through the mouse-algal-wet oxidizer system a simulation model has been developed, an adaptation of a related mass flow model based on humans, wheat and a wet air oxidation reactor (WAO). Elaboration of the model into algorithms has not been completed although the development of model structure has enhanced analysis of experimental data and, once completed, will be useful for predicting the behavior of the experimental system. Initially, the experimental data will be used to validate the model. Once validated, the model can be used in place of the experimental system to more rapidly determine the flow of mass under a variety of operating conditions. The basic structure of the model (Figure 6) is explained in detail below.

An important consideration in the development of the model has been the degree of complexity incorporated into it. There are several levels of complexity which have been considered. These include the compartment level (i.e. mouse and its environmental conditions), the organism level, the organ system level, the cellular level, the biomolecular level and the atomic level. Increasing the detail of the model to include the atomic interactions associated with biochemical reactions is considered to be too detailed. Similarly the compartment level alone is too general to yield useful information on the flow of materials through the system. It was determined that the compartment level containing the biomolecular level as a subsystem is most appropriate. The biomolecules that are included are carbohydrates, proteins and lipids. Other than accounting for C,O,N,H in such biomolecules, there may be no need to distinguish these compounds further in terms of detailed biochemical pathways. In fact, some areas of the model may not include detail at this level. For example, for certain segments of the model it may be necessary to include only the living component as a whole and not to break it down into specific molecules.

Model structure will be illustrated by tracing the flow of carbon. The model has been broken into segments to clarify the interactions occurring within each compartment. Figure 7a shows the flow of carbon through the mouse compartment. Carbon is introduced into the system from either mouse food or algal cells in the forms of carbohydrate, lipid and protein. Once eaten by the mouse, the metabolism of carbohydrate and lipid yields energy by conversion of simple sugars to CO_2 and H_2O in the presence of O_2. Additionally, the fats and sugars can be stored by the mouse. The carbon in proteins is used in maintenance of the mouse, the net flow may

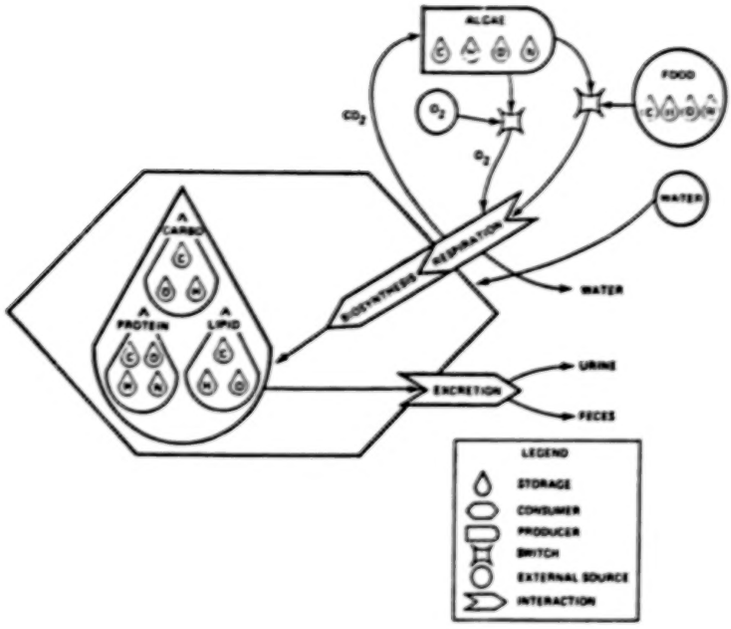

Fig. 7a. Simulation model, mouse compartment.

be set to zero or to whatever value experimental data suggests. Thus carbon introduced to the mouse is 1) metabolized to CO_2 and exhaled, 2) excreted in feces and urine or 3) retained in the mouse.

Table 1 shows the mouse mass balance which has been calculated manually from information contained within the model and from experimental data. The data was collected in two ways; values for CO_2 consumption and O_2 production were calculated from the slopes of RQ measurements made within the gas-closed mouse system, values for food and water consumption and fecal and urine production were obtained from metabolic cage experiments conducted outside the bell jar. The value for water vapor exhalations was not measured and thus was calculated from the O, H values remaining after all other inputs and outputs were balanced. The ratio of O to H was very close to the expected 8:1 ratio and the assumption was made that this remaining mass must be the water vapor content.

TABLE 1 Mass Balance of a Dwarf Mouse
(All values in grams/mouse/day)

	TOTAL	CARBON	HYDROGEN	OXYGEN	NITROGEN
OUT					
Urine Solids	0.1319	0.0267	0.0070	0.0250	0.0357
Urine Water	0.7300	-	0.0803	0.6497	-
Fecal Solids	0.1911	0.0684	0.0101	0.0591	0.0088
Fecal Water	0.3549	-	0.0390	0.3159	-
Water Exhaled	2.102	-	0.2393	1.914	-
CO_2 Exhaled	2.19	0.597	-	1.593	-
Subtotal	5.7871	0.6921	0.3757	4.534	0.0445
Storage (in mouse)	0.1760	0.1206	0.0133	0.079	0.0032
Total Out	5.9531	0.7227	0.3890	4.633	0.0477
IN					
Food	1.684	0.7447	0.1051	0.8299	0.0674
Water	3.660	-	0.3948	3.265	-
O_2 Consumption	1.810	-	-	1.810	-
Total In	5.974	0.7447	0.5979	4.8249	0.0674
Difference	0.021	0.022	0.2089	0.0	0.0197
Percent Recovery	99.65	97.05	97.8	100.0	70.8

The high percent recovery seen in Table 1 indicates that the mouse system demonstrates closure for the primary bioelements. Carbon and hydrogen flows show a 97% recovery; the 100% recovery of oxygen is found because the excess oxygen was all placed in the water vapor column and a stoichiometric amount of hydrogen was added to the hydrogen value. The 71% recovery of nitrogen is lower than expected. Possible sources of loss include the bacterial denitrification of urine and fecal urea to N_2, the loss of urine in the collection process by either volatilization or adherence to surfaces, and variation of solids contents in both urine and feces between different experimental runs.

Returning to the carbon trace through the system, the CO_2 exhaled by the mouse is introduced to the algae as its sole source of carbon (Figure 7b). Algal photosynthesis then converts the CO_2 into simple sugars and O_2 in the presence of water and light. Biosynthesis of the sugars to lipids and proteins may be incorporated into

the model, or the model can be run using only an algal biomass pool in place of the more specific biomolecules. Theoretically the algal biomass may then be recovered for use as a mouse food. Waste algal biomass may also be directed to the waste oxidizer.

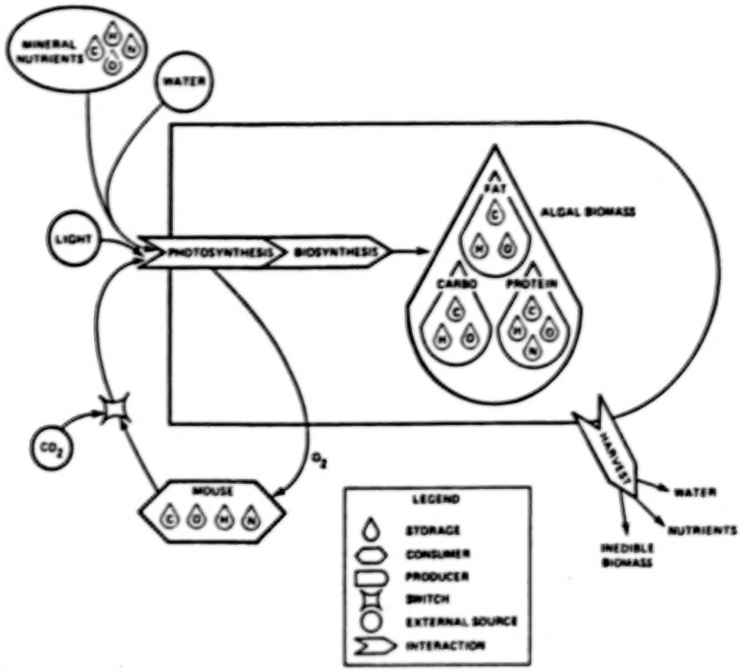

Fig. 7b. Simulation model, algal compartment.

The WAO receives carbon from the mouse feces and oxidizes it into CO_2, low molecular weight organic acids (primarily acetic) and CO in the presence of O_2 (Figure 7c). O_2 was supplied from a gas cylinder although theoretically it could be supplied from the algal compartment. When the carbon from mouse feces has been oxidized to CO_2 and this CO_2 is then metabolized to algal biomass, the cycle of carbon through the system is complete. Similar paths may be traced for oxygen, nitrogen and hydrogen so that an overall system balance for the major biomolecules may be observed.

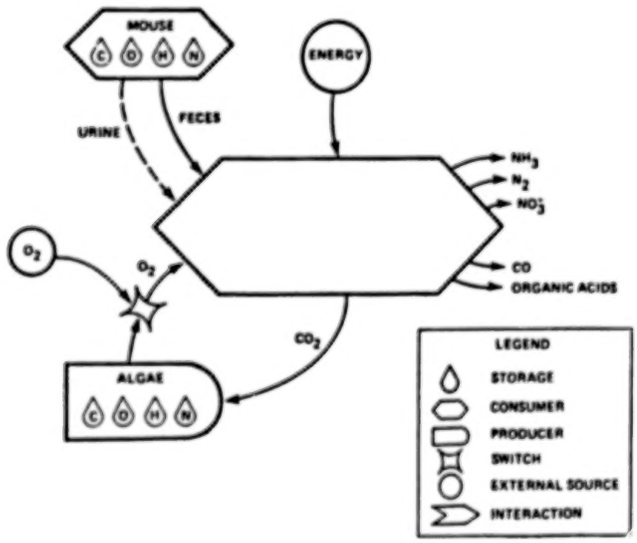

Fig. 7c. Simulation model, wet air oxidizer compartment.

Table 2 shows the mass balance of a single wet-air oxidation run using mouse feces. The excess carbon probably results from either variations in the percent carbon in feces or inaccuracies in the measurement of carbon streams in WAO effluent. The excess hydrogen in the system was assumed to be forming combustion water and thus was set to full recovery. The oxygen recovery is deceiving because of the large excess of oxygen used in the WAO process. Looking at the total oxygen used in the oxidation (1.80 g) and the recovery of only 1.03 g indicates a sink for oxygen which is not yet explained. Once again, nitrogen exhibits a low recovery (86%) which may due to difficulties in measuring small quantities of nitrogen compounds or variation in the nitrogen content of feces.

TABLE 2 Wet Air Oxidation Mass Balance
(All values in grams)

	TOTAL	CARBON	HYDROGEN	OXYGEN	NITROGEN
OUT					
Oxygen (g)	22.33	-	-	22.33	-
Carbon Dioxide (g)	0.7806	0.2129	-	0.5677	-
Carbon Dioxide (aq)	0.0118	0.0032	-	0.0086	-
Organic C (aq) (acetic acid)	0.1720	0.0460	0.0115	0.0917	-
Carbon Monoxide	0.2000	0.0857	-	0.1143	-
Nitrogen (g)	0.00444	-	-	-	0.00444
Ammonia (aq)	0.0361	-	0.0084	-	0.02968
Nitrate (aq)	0.00155	-	-	0.0012	0.00035
Organic N(aq)	0.00241	-	?	?	0.00241
Combustion Water	-	-	0.0311	0.2488	-
Precipitate (as Calcium Phosphate)	0.0007	-	-	0.045	-
Total Out	23.82	0.3788	0.049	23.61	0.0369
IN					
Feces	0.934	0.334	0.049	0.389	0.043
Oxygen	24.33	-	-	24.33	-
Total In	25.26	0.334	0.049	24.82	0.043
Difference	1.44	-0.0348	0.0	1.057	-0.0061
Percent Recovery	94.3	110.9	100.0	95.7	85.8

To change the steady-state flow balance of Figure 7 to a dynamic model, equations must be developed that express the various flows in terms of the state variables, i.e., the various storages of algal and mouse biomass, atmospheric CO_2, O_2, H_2O, light and nutrients. The simulation model being developed allows elemental balances to be calculated from experimental data. When the model is validated it will be useful to observe the changes in mass flow as conditions are varied. Thus, for example, the effects on the system of a doubling of the CO_2 input from the mouse compartment may be done completely by the model without having to actually add a mouse to the experimental system. The storage and flow of elements will be much easier to study with a well validated model than with the experimental system upon which it is based. As the model predicts interesting system behaviors, experiments may be conducted to verify model results and observe actual system behavior.

Table 3 shows one example of an overall system mass balance used to determine the accumulation and/or depletion of bioelements. The lack of complete experimental data, compounded by the non-integrated nature of the experimental system, makes it difficult to make accurate conclusions about element balance within the system. Data required includes; measurement of water vapor exhalations, recycling of urine, measurement of combustion water produced in the WAO and use of algal biomass as mouse food.

TABLE 3 Mass Balance of the Mouse-Algal-WAO System

MOUSE		
	RQ = 0.975	
	Start with:	0.5490 g feces
	which equates to:	2.190 g carbon dioxide produced
		1.610 g oxygen consumed
WAO		
	Requires:	0.802 g oxygen (to oxidize feces)
	Yields:	0.4580 g carbon dioxide
		0.1838 g carbon monoxide
		(+ 0.0068 g oxygen = 0.1838 g carbon dioxide)
		0.03633 g nitrogen
		(+ 0.1245 g oxygen = 0.1509 g nitrate)
	Total Oxygen Consumption:	2.60 g oxygen (1.610 + 0.0068 + 0.1245 + 0.802)
ALGAE		
	Requires:	2.829 g carbon dioxide (2.190 + 0.4580 + 0.1838)
	Produces:	3.070 g oxygen (AQ = 0.97) experimental
		3.110 g oxygen (AQ = 0.975) theoretical

The data shown in Table 3 indicates that if the AQ is less than the RQ there will be a net increase of O_2 in the system. This agrees with theoretical calculations /3/. If the AQ is equal to the RQ there appears to be a net loss of O_2 although a balance would be expected. The reason for this discrepancy is probably due to the formation of combustion water in the WAO. There is a significant loss of O_2 from the WAO mass balance

which, if completely accounted for, would probably balance the O_2 in the AQ equal to RQ scenario of Table 3. When looking at the whole system mass balance, the algal AQ must actually balance with the mouse RQ as well as the WAO RQ (which would equal the ratio of CO_2 produced/O_2 consumed).

The data in Table 3 exhibits the amount of NO_3^- which would be obtained from the oxidation of the specified amount of feces, assuming the complete reduction of all nitrogen to NO_3^-. This value could then be compared to the amount of NO_3^- required by the algal cultures to support one mouse for one day. Again, data is lacking to make this complete comparison because of the non-integrated nature of the system. However, it is clear that such analyses could be made with a combination of the simulation model and system modifications. Calculation of such balances at this time is not useful because of the inaccuracies introduced by the nature of the system. More accurate analysis will be possible with the stoichiometric equations contained in the simulation model and data acquired from integrated subsystems.

CONCLUSIONS

The operation of this experimental system has helped to elucidate issues associated with the development of bioregenerative life support (BLS) systems. The issues raised are of two varieties. One is the analysis of the role which algae will play in BLS systems. The second variety is concerned with the way in which algal systems will be designed and operated. The development of a functional BLS system will rely on numerous iterations of subsystems, the pre-prototype air revitilization system described here is one step in that process.

Both the positive and negative attributes of the role which algae may play in BLS systems have been outlined in /8/. Operation of this system demonstrated that algae can; be controlled by manipulation of inherent characteristics of the organisms, and satisfy respiratory gas requirements. We also encountered some of the same difficulties anticipated of space-based systems. The inability to use algal-derived food prevented mass closure of the experimental system and the presence of bacterial contaminants raised questions about the long-term stability and reliability of algal cultures.

The operation of the experimental system demonstrated that algae are capable of providing air revitalization under controllable conditions and by use of endogenous algal characteristics. Problems associated with bacterial contamination can be dealt with by understanding relative population dynamics and compensating for bacterial respiration by increased algal photosynthesis. Determination of appropriate material reservoirs can be explored experimentally and will lead to proper scaling of buffers. Development of the simulation model will aid in examining issues related to mass balance, subsystem integration and overall system performance (stability and reliability).

Application of these principles to a human-scale system will require further experimentation to; define optimal algal environmental conditions, engineer functional systems which require minimal human attention and produce sufficient algal biomass to satisfy the respiratory and nutritional requirements of humans.

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NON-CONVENTIONAL APPROACHES TO FOOD PROCESSING IN CELSS. I-ALGAL PROTEINS; CHARACTERIZATION AND PROCESS OPTIMIZATION.

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ABSTRACT

Protein isolate obtained from green algae (Scenedesmus obliquus) cultivated under controlled conditions was characterized. Molecular weight determination of fractionated algal proteins using SDS-polyacrylamide gel electrophoresis revealed a wide spectrum of molecular weights ranging from 15,000 to 220,000. Isoelectric points of dissociated proteins were in the range of 3.95 to 6.20. Amino acid composition of protein isolate compared favorably with FAO standards. High content of essential amino acids leucine, valine, phenylalanine and lysine makes algal protein isolate a high quality component of CELSS diets. To optimize the removal of algal lipids and pigments supercritical carbon dioxide extraction (with and without ethanol as a co-solvent) was used. Addition of ethanol to supercritical CO_2 resulted in more efficient removal of algal lipids and produced protein isolate with a good yield and protein recovery. The protein isolate extracted by the above mixture had an improved water solubility.

INTRODUCTION

There is a need for suitable feeding systems for extended manned space missions. The potential utilization of algal biomass in a non-conventional food supply in space, is the subject of our study. Unique multifunctional characteristics of algae such as high photosynthetic activity, lack of need for organic carbon and nitrogen in the growth media and the ability to control algal chemical composition by varying cultivation conditions make algae an attractive system for biological regeneration of the CELSS environment /1,2,3,4,5,6,7,8,9/. Consumption of untreated and unpurified algal biomass, due to associated physiological problems (e.g. nondigestible components of cell wall and disturbing carbohydrates, excess nucleic acids, unknown toxins and allergens) and poor flavor or color limits the amount of algae in the diet. Conversion of algal biomass into physiologically and organoleptically acceptable components requires suitable methodology. Previously developed methods eliminated or largely reduced three known undesirable components of algae (cell walls, nucleic acids, and pigments) and gave an "algal protein isolate" with a reasonably high yield /10/.

Green algae (Scenedesmus obliquus) grown in Constant Cell Density Apparatus, under controlled conditions were used in this study. They were obtained through the courtesy of Drs. Richard Radmer and Paul Behrens of MARTEK Corp. Columbia, MD. Figure 1 shows the stepwise procedure for preparation of various fractions from algae. Major nutritional components of the algae flour (freeze-dried ruptured cells) were determined. Upon cell rupture and removal of the cell wall, the nucleic acid content of the algae was reduced by 92%, using an enzymatic procedure. This increased the safe consumption level of algal protein concentrate (fraction #6) from 20g to approximately 250g per day and for algal protein isolate (fraction #8A) from 15g to 192 g per day. Generally accepted safe level of nucleic acids intake in human is 2g per day /11/. Ethanol extraction of algal pigments (mostly chlorophyll A) from the protein concentrate (fraction #6) resulted in removal of most of the green color and consequently the algal protein isolate (fraction #8A) had a "light olive" color upon freeze-drying. Removal of the pigments enhanced the color and flavor, but caused protein denaturation and insolubility of the final product.

In order to further improve the quality of algal proteins we have experimented with the removal of algal pigments and lipids using supercritical fluids. The results are reported here.

Characterization of isolated algal proteins was a major part of our recent study. This knowledge can be useful in designing further processing and fabrication of algal proteins

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as a food component in CELSS. The present paper also reports the results of electrophoretic studies and amino acid analyses of algal proteins.

METHODOLOGY

Preparation of algal protein fractions

Green algae (Scenedesmus obliquus) were grown in Constant Cell Density Apparatus (CCDA). Conditions were: Algal density in medium: 0.55 mg dry weight/ml.; 32°C; pH= 7.0; nitrogen source: KNO_3 2.0g/l; carbon source: 2% CO_2 in air. The algae were grown, harvested, and supplied to us by Martek Corp. (Columbia, MD).

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to determine the molecular weights (M.W.) of algal proteins. Freeze-dried algal protein concentrate (fraction #6) was used. The experimental details of SDS-PAGE were reported previously /12/.

Isoelectric focusing (IEF)

IEF in polyacrylamide gel was used to determine the isoelectric points (pI) of algal proteins. Ampholine pH 3-10 was used to produce pH gradient across the gel. The IEF conditions were as previously reported /12/. Freeze-dried algal protein isolate (fraction #6) was used in this study.

Amino acid analysis

Amino acid composition of algal protein isolate was determined using a recently developed pre-column derivatization method which has been originally demonstrated by Koop, Morgan, Tarr and Coon /13/ for analysis of free amino acids and later modified by Bidlingmeyer, Cohen and Tarvin /14/ for application to acid-hydrolysates of proteins.

The method which is called "Pico-Tag" is based on derivatization of amino acids with phenylisothiocyanate (PITC). PITC reacts with free amino acids to yield phenylthiocarbamyl (PTC) amino acid, which can then be separated on a reversed-phase HPLC column. Strong UV absorbance of PTC-amino acids (254 nm) makes it possible to detect quantities as small as 1 picomole /14/. Because PITC forms the same chromophore with primary and secondary amino acids no extra treatment is necessary for detection of proline. Excellent reproducibility, derivatives stability (6-10 hrs at R.T.) and rapid analysis (12 min.) of amino acids made the Pico-Tag the method of choice for our purpose.

Upon HCl hydrolysis destruction of Trp, conversion of Asn and Gln to ASP and Glu , also oxidation of cysteine to cystine and consequently partial destruction of cystine occur /15/. Hence, in order to obtain complete amino acid profile, algal protein isolate (fraction #8A) was treated in 3 different ways: 1) HCl hydrolysis and PITC derivatization to detect all PTC-amino acid derivatives except for Cys and Trp, 2) Performic acid oxidation followed by HCl hydrolysis and derivatization to quantify the amount of cysteine in the form of cysteic acid, and 3) methane-sulfonic acid hydrolysis followed by derivatization to obtain the amount of tryptophan. Amino acid standard used was Pierce standard H. Least square treatment of data and calculated regression lines were used for quantification of amino acid content of algal proteins.

Supercritical Fluid Extraction

Supercritical fluid extraction of algal pigments and lipids was carried out in two steps. The 1st extraction was with supercritical carbon dioxide (SC -CO_2) and the 2nd extraction was with carbon dioxide and anhydrous ethanol (22%) as a co-solvent. Freeze-dried algal protein concentrate (fraction #6), 4.86g was charged alternatively between layers of glass wool to an extraction vessel, a 1.8 cm diameter X 30 cm long stainless steel tube (Autoclave Engineers, Inc.), and connected to the system shown in Figure 2; the glass wool served to keep the algae powder from compacting during passage of gas through the extractor. Carbon dioxide (Airco, Inc., Grade 2.8) was supplied at about 87 bar pressure and 313°K to the suction side of a double-end diaphragm compressor (Superpressure, Inc.) and was compressed to the measurement pressure. The pressure was controlled by a back-pressure regulator (Circle Seal, BPR) which diverted the bulk of the compressed gas from the surge tank back to the suction side of the compressor resulting in an almost pulse-free flow of gas to the extractor. The high-pressure gas passing downstream of the compressor was heated in a tube preheater to about 338°K and was passed through the extraction vessel which was maintained at 338°±2 K by a temperature indicator/controller which measured the temperature via an iron constantan thermocouple (Superpressure, Inc.) positioned in the bed of algae powder and which regulated power to a heater (Glascol tapes) on the extractor.

The solution (consisting of carbon dioxide and dissolved materials) leaving the extraction vessel was passed through a heated, flow-regulating, pressure let-down valve and was expanded to ambient pressure. The materials which were dissolved by the gas passing through the extraction vessel precipitated during the pressure-reduction step and were separated from the gas in a U-tube collector (Kimble, 200 mm) whose exit junction was fitted with a glass wool filter to prevent fine-particle solids from passing through the tube; a second U-tube with a more tightly packed glass wool filter was positioned downstream of the first collector and served to trap any fine particles which might have passed through the first filter.

The ambient gas leaving the collection system passed through a rotameter, (Fischer-Porter, Inc., Series 10A 35) for flow rate measurement and through a dry test meter (Singer, Inc., DTM-200) for flow volume integration. The flow rate of the carbon dioxide through the extractor was maintained at 3 SLPM (standard liters per minute). A total of 0.66 g of green semi solid materials, SC-CO_2 extract (fraction #7B) was collected (in the U-tube) during the passage of 720 g of carbon dioxide at 380 bar pressure. After the extraction with carbon dioxide a fraction of the charge of algae (SC-CO_2 residue, fraction #8B) was removed from the vessel to be used for solubility tests, the vessel was resealed and connected to the system. The 2nd extraction was carried out with SC-CO_2 at 380 bar pressure at 338° K, with anhydrous ethanol added as a co-solvent. It was pumped into the system at the entrance to the extractor. The ratio of ethanol to carbon dioxide was 100 to 360 g (22%). The SC-CO_2 + EtOH extract (dark green ethanol solution) was collected in a container (fraction #7C). The SC-CO_2 + EtOH residue (fraction #8C) was used for solubility test (Figure 3).

Solubility Test

Algal protein isolate (fraction #8A), SC-CO_2 residue (fraction #8B) and SC-CO_2 + EtOH residue (fraction #8C) were used. To 100 mg of each fraction, 10 ml, 0.05 M phosphate buffer, pH 8.0 (based on pH-solubility profile of plant proteins, Wolf /17/) was added. The samples were mixed and stored at room temperature for about 1 hr. They were then centrifuged at 15,000 RPM for 10 min. at 2° C. The supernatant was collected and the pellet was washed with another 2 ml of phosphate buffer and recentrifuged. The wash was added to supernatant (kept at 5° C) and the pellet was freeze-dried (Virtis Research Equipment, Gardiner, NY). Protein concentration of the supernatants and the pellets were determined using the micro-kjeldahl method A.O.A.C. /18/. Solubility was defined as:

$$\% \text{ Solubility} = \frac{\text{Protein in supernatant (mg)} \times 100}{\text{(sum of protein in supernatant and pellet) mg}} \qquad (1)$$

Absorption Spectra

Absorption spectra of SC-CO_2 extract (fraction #7B) and SC-CO_2 + EtOH extract (fraction #7C) in the range of 700 to 240 nm were obtained and compared with that of fraction 7A, which is the boiling-ethanol extract. Since SC-CO_2 extract (fraction #7B) is in the form of semi solid materials, an aliquot was dissolved in hexane, filtered (Millex-SLSRO25NS) and used for this study.

Analysis of Algal Lipids

Isolation of algal lipid components (neutral, glyco and phospholipids) using sequential solvent extraction, gel filtration and thin layer chromatography, as well as determination of fatty acid composition using GC is underway. SC-CO_2 and SC-CO_2 + EtOH extracts (fractions #7B and 7C) and the corresponding residues (fractions #8B and 8C) will be analyzed.

RESULTS AND DISCUSSION

Molecular Weight Determination

Literature on molecular weights of algal proteins is limited. Lee and Picard /19/ studied the electrophoretic pattern of Oocystis algal proteins using three systems: Tris-glycine pH 8.7, SDS-gel and SDS-gel with 2-mercaptoethanol. Using the SDS-gel along with dissociating agents they observed a wide spectrum of molecular weights (M.W.) ranging from 12,500 to 250,000. In our study using SDS-PAGE we also obtained wide range of M.W. for Scenedesmus obliquus proteins. Figure 4 shows the electrophoretic pattern of protein concentrate (fraction #6). The M.W. of proteins ranged from 15,000 (band #20) to 220,000 (band #1). Major protein bands correspond to bands # 7, 14, 15, 18, 19 and 20 with M.W. of 55,000, 30,000, 28,000, 18,000, 16,500 and 15,000 respectively. Band #21 corresponded to chlorophyll and other algal pigments (M.W. below 1,000) which migrated to the front of the gel. A comparison of SDS-PAGE pattern of protein isolate obtained from ethanol extraction (fraction #8A) with that of protein isolate resulting from supercritical fluid extraction (fractions #8B and 8C) will enable us to detect the extent of changes such as oligomerization and/or partial fragmentation of treated algal proteins.

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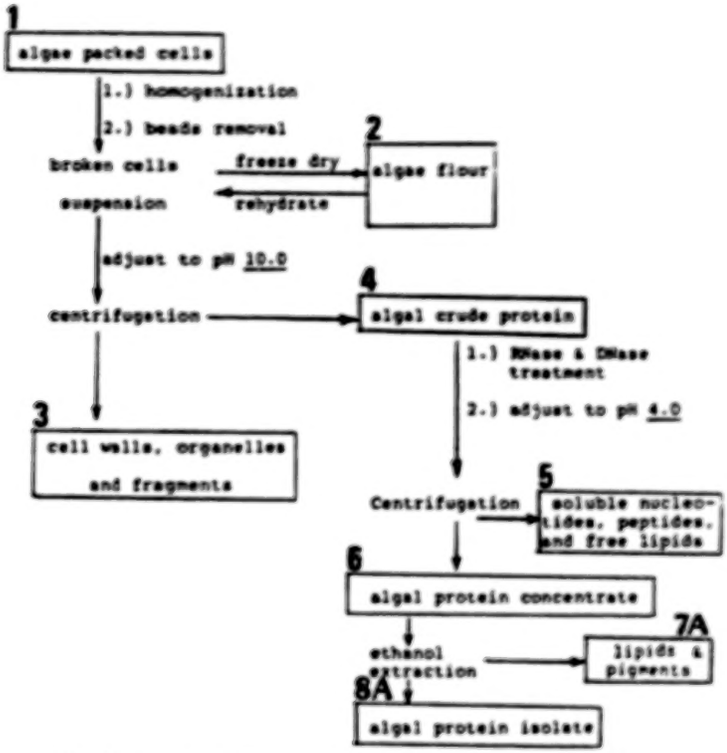

Fig. 1) Stepwise procedure for preparation of various algae fractions.

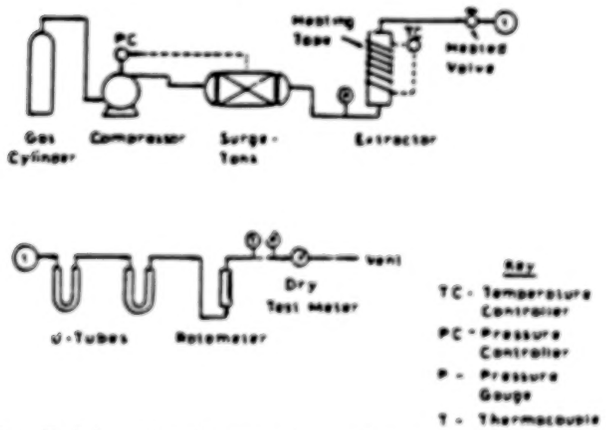

Fig. 2) Schematic diagram of experimental for supercritical fluid extraction (from /16/).

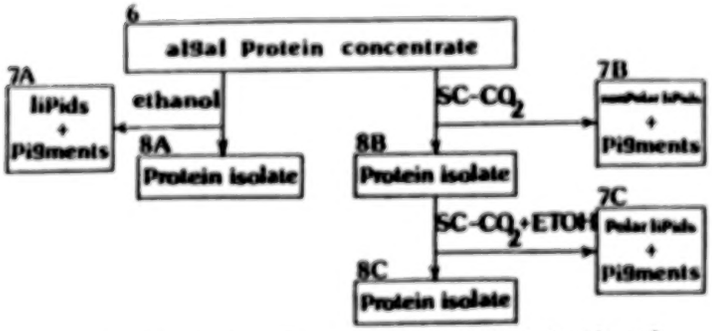

Fig. 3) Procedure for extraction of algal lipids and pigments using supercritical fluids (at about 380 bar pressure and 338°K).

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Isoelectric Point Determination

There is no report available on isoelectric points of algal proteins. The IEF pattern of algal proteins and pH profile of IEF gel for determination of the PI's of focused proteins are shown in Figure 5. The isoelectric points of the algal proteins ranged from 3.95 (band # 15) to 6.20 (band #2). Major protein bands corresponded to bands #7, 9, 11 and 13 with PI's of 5.45, 5.20, 4.85 and 4.35 respectively. Band #1 corresponded to chlorophyll and other pigments which did not migrate in the IEF gel.

Amino Acid Composition

Nutritional value of proteins is primarily determined by their amino acid composition. Hence amino acid profile of algal protein (Scenedesmus obliquus) was studied and aminograms were obtained (Figure 6).

The amino acid composition of algal proteins compared favorably with FAO standards, Table 1. Analysis of amino acid composition revealed high content of leucine, valine, phenylalanine and lysine and low content of methionine and tryptophan. (The high values for cysteine contrary to reported low values in the literature is because we analyzed cysteine in the form of cysteic acid which yielded in about 70% more compared to values we obtained for cystine).

There is no data reported on amino acid composition of Scenedesmus obliquus cultivated under controlled conditions (CCDA). However, there are reports on amino acid composition of Scenedesmus obliquus cultivated under open air conditions according to German technique of Dortmund /20, 21, 22, 23/ cultivation ponds /24/ and unspecified conditions /5/. The comparison of the aminograms from Scenedesmus obliquus cultivated under different conditions revealed a similar pattern of high content of valine, leucine and lysine and low content of methionine and tryptophan. Our results, showed relatively higher values for isoleucine, leucine and phenylalanine.

The nutritional value of green algae depends on digestibility which depends on pretreatment of algae and disruption of the cell wall. Low nutritional value is to be expected if algae is not sufficiently processed /25/. Nutritional value (biological value, BV, digestibility, D, net protein utilization, NPU, and protein efficiency ratio, PER) of pretreated Scenedesmus obliquus cultivated under open air conditions (Dortmund technique) has been studied in a series of different and independent investigations in animals and also in human volunteers. The results repeatedly and unequivocally confirmed high nutritive value of algal proteins /25, 26, 27, 28/. Drum-dried Scenedesmus obliquus was studied as a diet component in rats. High nutritional value due to improved protein digestibility (as a result of cell disruption) was reported, Table 2 /30, 31/. Fortification of wheat and rice with proteins from Scenedesmus acutus in rats' diets resulted in significantly higher PER value than cereal diet alone /32/. They also concluded that Scenedesmus proteins supplemented wheat proteins to a greater extent than rice.

In summary, algal proteins, because of their high content of essential amino acids (valine, leucine, lysine and phenylalanine) upon cell wall rupture and removal of undesirable components, can significantly improve the nutritional value of other plant proteins e.g. cereals (wheat, rice and corn with lysine as their limiting amino acid), legumes, etc. and serve as a high quality component of CELSS diets. Wheat and soybeans are considered as two sources of macronutrients in CELSS diet scenario /33/.

Supercritical Fluid Extraction

In our previous study boiling ethanol was used to remove pigments and lipids. Ethanol treatment of algal proteins (regardless of extraction temperature) improves the color and flavor of the isolate, but causes denaturation and consequently aggregation of algal proteins with loss of solubility. Supercritical fluids are receiving increasing attention as extraction solvents. Because of their pressure-dependent dissolving power properties often display the fractionation ability of multicomponent solutes /34/. Supercritical carbon dioxide (SC-CO_2) has been considered by many investigators as the ideal fluid for extraction and separation processes. It is reported to behave very much like hydrocarbon solvent with very low polarizability /35/. Therefore, the use of SC-CO_2 for extraction of lipids or lipid soluble materials while giving comparable yields offers several advantages.

Organic solvents are flammable and explosive. They might also contain traces of higher boiling fractions that may be left in the extract and pose a potential health hazard /36/. On the other hand carbon dioxide is nontoxic, nonflammable and rather easily separatable from the extracted materials.

There are an increasing number of reports on replacement of lipophilic extraction by SC-CO_2. Extractions of oil from corn /36, 37/, soybeans /38, 39, 40/, cottonseed /41/, lupine seeds

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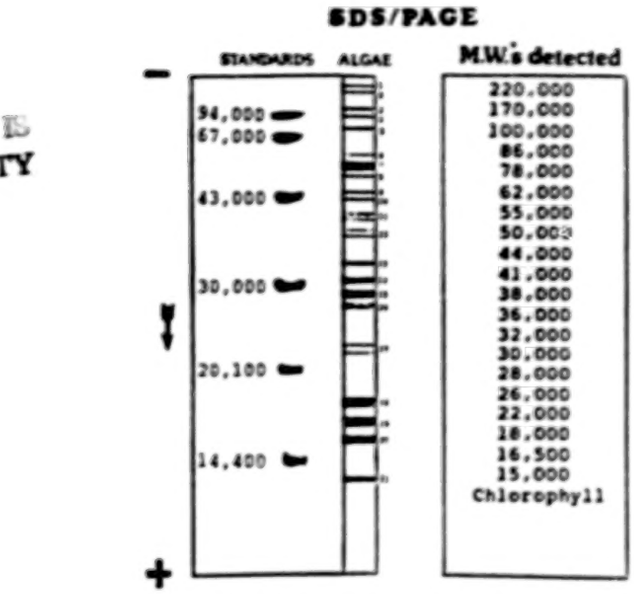

Fig. 4) SDS-PAGE pattern of algal protein concentrate (fraction #6).

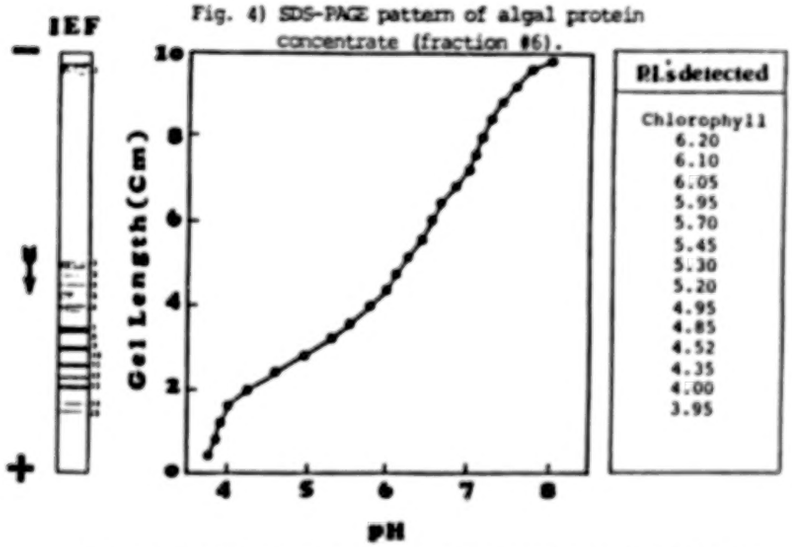

Fig. 5) IEF pattern of algal protein concentrate (fraction #6) and pH profile of IEF gel for determination of pI of focused proteins.

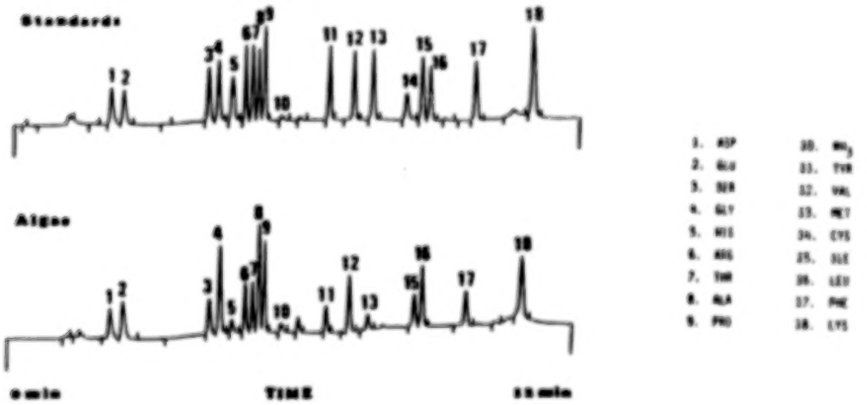

Fig. 6) HPLC chromatograms of amino acid standards (Pierce standard H) and of algal protein isolate (fraction #8A)

/42/ and animal oils and fats /43/ using supercritical CO_2 has been reported.

Comparative study on the storage ability of defatted corn germ flour using hexane and SC-CO_2 showed significant reduction of peroxidase activity (apparently as a result of protein denaturation under the extraction conditions, 344 to 551 bar pressure and 323° K) and high flavor quality upon accelerated storage tests for SC-CO_2 extracted corn germ flour /37/. Similar study on oil extraction of soybean flakes using SC-CO_2 (730 to 854 bar pressure, 353-373° K and 5-13% moisture) and hexane also resulted in better flavor quality and shelf life for SC-CO_2 defatted soybean flour /40/. In a comparative study on oxidation stability of "oil" extracted from soybean flakes using either SC-CO_2 at 344 bar and 323° K or hexane, SC-CO_2 extraction yielded a product comparable to a hexane-extracted degummed soybean oil. Phosphatides were essentially absent in SC-CO_2 and this may be the reason for lower oxidative stability of SC-CO_2 extracted soybean oil /39/.

There is no report on supercritical extraction of algal lipids and/or pigments. The extraction of algal protein concentrate (fraction #6) using (SC-CO_2) resulted in removal of green semi solid materials believed to contain nonpolar lipids and lipid-soluble pigments (such as chlorophyll and carotenoids). Chlorophylls are classified as lipid- and water-soluble and carotenoids as lipid-soluble pigments /44/. The yield of SC-CO_2 extract (fraction #7B) was 7%. The absorption spectra of fraction #7B (dissolved in hexane) indicated the predominance of chlorophyll A (with absorption maximum, λ_{max} at 410 nm) and some other minor peaks probably due to extracted carotenoids. The yield of SC-CO_2 residue (fraction #8B) which had an "olive green" color was 44.6%. Further extraction of protein isolate (fraction #8B) using SC-CO_2 and 22% ethanol resulted in a dark green ethanol solution believed to contain polar lipids and chlorophylls (fraction #7C) with the yield of 12%. The absorption spectra of SC-CO_2 + EtOH extract indicated mainly the presence of chlorophyll A and traces of chlorophyll B (λ_{max} at 470 nm). The absorption spectra of SC-CO_2 + EtOH extract (fraction #7C) were almost identical to those of ethanol extract (fraction #7A). The SC-CO_2 + EtOH residue (fraction #8C) had a "light olive" color similar to ethanol extract residue (fraction #8A). Yield of algal protein isolate obtained from SC-CO_2 + EtOH extraction was 33%. The remaining balance as compared to previously reported value for fraction 8A which was 36.6%, is due to the removal of 19% total lipids (nonpolar and polar) and pigments (fraction #7B and 7C) compared to 15% (fraction #7A). We believe that SC-CO_2 extraction resulted in more efficient removal of nonpolar lipids. Isolation and determination of lipid components (neutral, glyco and phospholipids) and fatty acid composition of various extracts (ethanol, SC-CO_2 and SC-CO_2 + EtOH) and the residues areunderway and will be reported in our next paper. Protein concentration of SC-CO_2 + EtOH residue (protein isolate 8C) was 69.1%, similar to the ethanol residue (protein isolate 8A) which was 70.5%. Table 3 shows the yield, protein concentration and recovery of various algal fractions.

Protein Recovery

Recovery of Oocystis algal proteins was studied by Lee and Picard /19/. Using successive ammonium sulfate fractionations they recovered 44% of algal proteins. The protein recovery increased to 66% when the fractionation was done in the presence of ascorbic acid and insoluble PVP (polyvinylpyrrolidone). The presence of endogenous proteolytic and oxidative enzymes may partially impair the recovery of plant proteins.

Phenoloxidase and peroxidase catalyze the reduction of O-diphenols to quinones; subsequently the quinones polymerize and form complexes with proteins thus impairing the solubility /19, 45, 46, 47/. The formation of these complexes is prevented by extracting the plant proteins in the presence of reducing agents (such as ascorbic acid) and by removing the phenols with substances such as PVP which forms hydrogen bonds with phenols. Nonspecific aggregation of plant proteins is also reported in the literature and believed to be the result of sulphydryl interactions /48/.

Solubility of Algal Proteins

Solubility of algal proteins is shown in Table 3. Under our experimental conditions, the solubility of algal protein (fraction #6) prior to removal of pigments and lipids was 63%. Upon extraction with ethanol the solubility decreased to 20% (protein isolate 8A). The solubility of SC-CO_2 residue (protein isolate 8B) was 45% and of SC-CO_2 + EtOH residue was 41%.

Partial protein denaturation as a result of "anhydrous" SC-CO_2 extraction (344 to 551 bar pressure and 323° K) of dry-milled corn germ flour /37/ and "humid" SC-CO_2 (730 to 854 bar, at 373° K and 5-13 % moisture) extraction of soybean flakes /40/ has been reported. Protein denaturation has been considered to be the result of high pressure extraction /37/. However, this partial denaturation resulted in reduction of peroxidase activity (heat-resistant oxidative enzyme) and consequently better storage stability of the extracted corn germ flour and soybean flakes. Weder /49, 50/ studied the effect of humid supercritical CO_2 and N_2 at 300 bar, 353° K and room temperature on the structure and amino acid composition of ribonuclease and lysozyme. The amino acid composition and TNBS-reactive lysine (trinitrobenzene

sulfonic acid) remained unaltered. Partial oligomerization (due to disulfides) and some fragmentation of protein molecules in protein extracted at 353° K was detected. No oligomers were detected using SDS-PAGE when extraction was at room temperature. The occurrence of the changes in the protein molecules was concluded to be the result of heating proteins in the presence of water regardless of the nature or pressure of the gas used. Tryptic digestion of the treated proteins indicated a better digestibility than untreated proteins.

In our system, decrease in the solubility of protein isolate (41%) as compared to protein concentrate (63%) could possibly be due to the extraction temperature (338° K). In our previous study on enzymatic reactions of polyphenol oxidase in supercritical fluids the enzyme was found to be catalytically active at 344 bar, 307° K and 309° K /51/ indicating that no protein denaturation has occurred due to the exposure to supercritical fluids.

In summary: SC-CO_2 + EtOH extraction of green algae resulted in : 1) more efficient removal of algal lipids and pigments (19% vs 15% ethanol extract). 2) Fractionation of nonpolar and polar lipids. 3) Protein isolate with a good yield and protein recovery. 4) Increased solubility of the protein.

The above results suggest that supercritical fluid extraction is a useful extraction method to be used in the space habitats.

Acknowledgements:

The authors are grateful to Drs. Richard Radmer and Paul Behrens (Martek Corp., Columbia, MD) for supplying them with the algae used in this study. This work was supported by the NASA CELSS Program.

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Table 1 Amino Acid Composition of Algal Protein Isolate (Fraction #BA)

(g/100g protein)

Amino Acid	FAO Standard	Scenedesmus obliquus
ASP		8.0
Glu		8.3
Ser		3.6
Gly		6.1
His		1.8
Arg		5.8
Thr*	4.0	4.6
Ala		8.3
Pro		5.8
Tyr		3.5
Val*	5.0	7.3
Met*	2.7	2.2
Cys		7.0
Ile*	4.0	5.0
Leu*	7.0	11.1
Phe*	3.4	6.0
Trp*	1.0	a
Lys*	5.5	5.7
%Essential	32.6	42.0

*Essential amino acid. a. not reported here.

Table 2 Nutritional Value of Algae in Comparison with Some Representative Food Proteins (from /29/).

	PER	BV	NPU	C.S.	limiting a.a.
Egg	3.8	87-97	91-94	100	none
Soybeans	0.7-1.8	58-69	48-61	69	S
Rice	1.9	75	70	57	Lys.
Corn	1.2	60	49-55	55	Lys.
Wheat	1.0	52	52	57	Lys.
Algae[a]	3.21	81	68	57[b]	Met. Trp.

(a), Drum-dried Scenedesmus obliquus (from /31/).
(b), from /51/.
PER, Protein Efficiency Ratio.
BV, Biological Value.
NPU, Net Protein Utilization.
C.S., Chemical Score.
S, Sulfer-containing amino acid.

Table 3 Yield (%), Protein Concentration (%) Protein Recovery (%) Solubility (%) of Various Algae Fractions Without Enzymatic Treatment.

Fraction #	Yield (%)	Protein Concentration (%)	Protein Recovery (%)	Solubility (%)
2 (algae flour)	100	52.6	100	---
4 (algal crude protein)	65	57.6	71.2	---
6 (algal protein concentrate)	51.6	53.4	52.4	63
BA (algal Protein Isolate)	36.6	70.5	49.1	20
BB ($SC\text{-}CO_2$ Protein Isolate)	44.6	66.5	56.4	45
BC ($SC\text{-}CO_2$ + EtOH Protein Isolate)	33.0	69.1	43.3	41

N88-12257

APPLICATION OF PHOTOSYNTHETIC N_2-FIXING CYANOBACTERIA TO THE CELSS PROGRAM

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ABSTRACT

The feasibility of using photosynthetic microalgae (cyanobacteria) as a subsystem component for the CELSS program, with particular emphasis on the manipulation of the biomass (protein/carbohydrate) has been addressed. Using factors which retard growth rates, but not photosynthetic electron flux, the partitioning of photosynthetically derived reductant may be dictated towards CO_2 fixation (carbohydrate formation) and away from N_2 fixation (protein formation). Cold shock treatment of fairly dense cultures markedly increases the glycogen content from 1% to 35% (dry weight), and presents a useful technique to change the protein/carbohydrate ratio of these organisms to a more nutritionally acceptable form.

INTORDUCTION

The use of biological components in the CELSS program as subsystems for air revitalization, waste processing or the production of food has been proposed for long-term space flight /1/. Employment of algae (particularly cyanobacteria), which generate biomass from relatively simple components (minerals and light) and their ability to fix atmospheric N_2, make them an attracive component for incorporation into the CELSS program. However, if use of cyanobacteria is envisaged as a major food source, manipulation of the composition of the biomass is required. Cyanobacteria (aptly described as single cell protein) are approximately 50% protein, with varying levels of carbohydrates, 1% (dry weight) in freshwater non-nitrogen fixers /2/, and up to 30% in some nitrogen fixing strains /3/. The average human nutritional daily requirement is for 20% protein and 50% carbohydrate (table I).

MATERIALS AND METHODS

Nostoc muscorum was grown in BG11 medium minus nitrate and Synechoccus 6311 was grown in KMC medium, in a 2 litre Bethesda Research Laboratories Airlift Fermentor at 30°C unless otherwise stated, 150 u $Es^{-1}m^{-2}$ light (using Bethesda Research Laboratories 2201 LB day light white 300-700nm) with an airflow rate of 2 litres/min, supplemented with 0.5% CO_2 . 200 ml aliquots were withdrawm daily, the fermentor volume made up by addition of 200ml of sterile medium. Cells were centrifuged at 10,000xg/10 min and resuspended to 2 ml in BG11 or KMC medium supplemented with 10 mM Tes buffer pH 7.0.

O_2 evolution was monitored polarographically in BG11 or KMC plus 10mM Tes pH7.0 with a cell density equivalent to 1-2 ug chlorophyll/ml. Nitrogenase activity was determined in whole filaments by monitoring acetylene reduction using a Varian Model 3700 gas chromatograph fitted with a Poropak T column. Cells (3 ug chlorophyll in 3 ml) were assayed in a 5 ml vial under air plus 10% (v/v) C_2H_2 in a shaking waterbath at 28° under 50u$Es^{-1}m^{-2}$ light.

Light intensities were measured using a Li-Cor inc. integrating quantum/radiometer/photometer Li-188B, with a Li-190SB quantum sensor. Glycogen was extracted and determined colorimetrically by the method of Van Handel /4/.

We have previously reported on the affects of salt shock on cellular glycogen content of a freshwater non-nitrogen fixing cyanobacterium,

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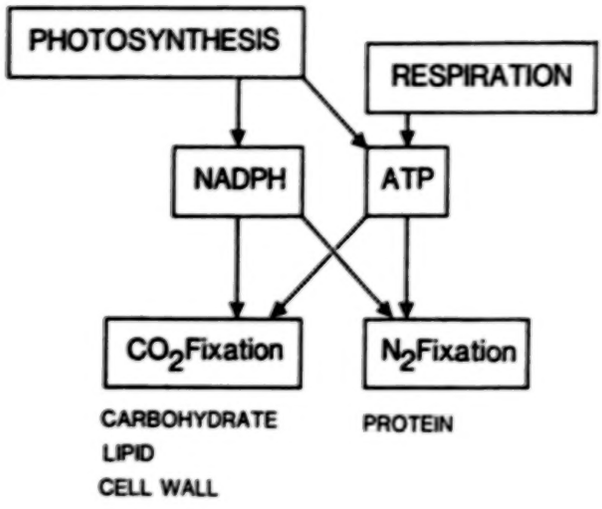

Fig.1 Scheme for the sources of reductant and ATP for CO_2 and N_2 fixation.

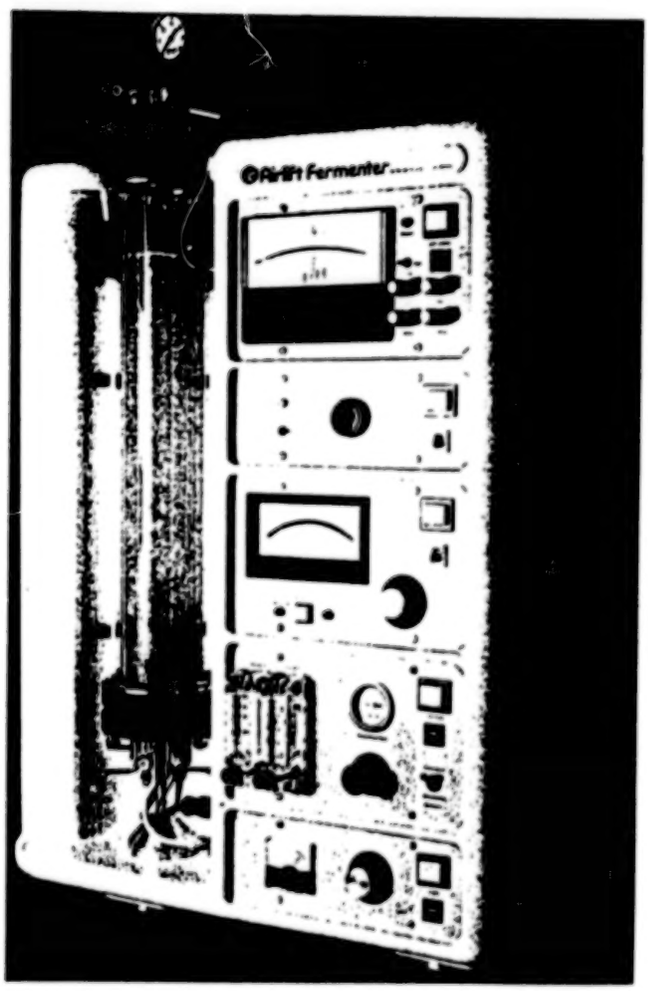

Fig.2 A 21 Bethesda Research Laboratory Air-Lift fermentor.

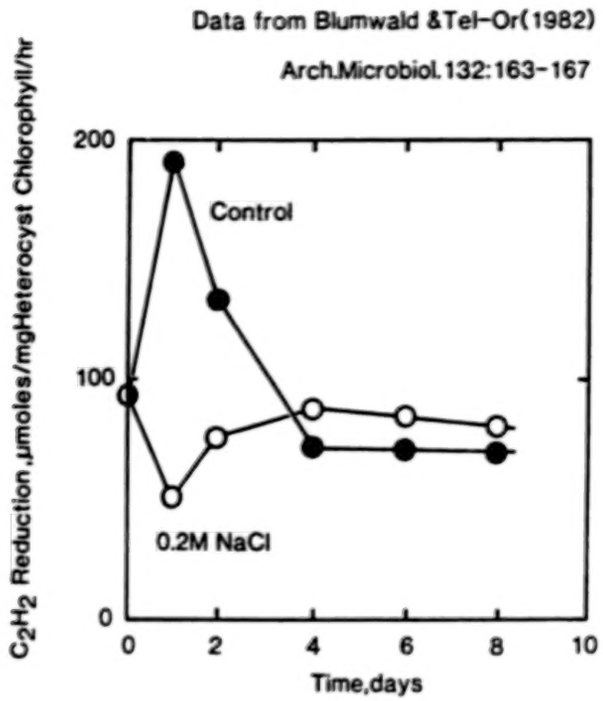

Fig.3 Nitrogenase activity during exposure to salinity of Nosoc muscorum.

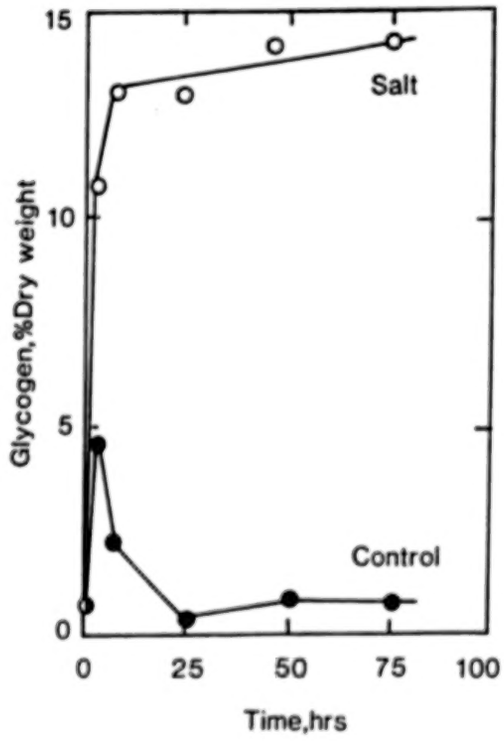

Fig.4 Cellular glycogen content of Synechococcus 6311 during growth under saline conditions.

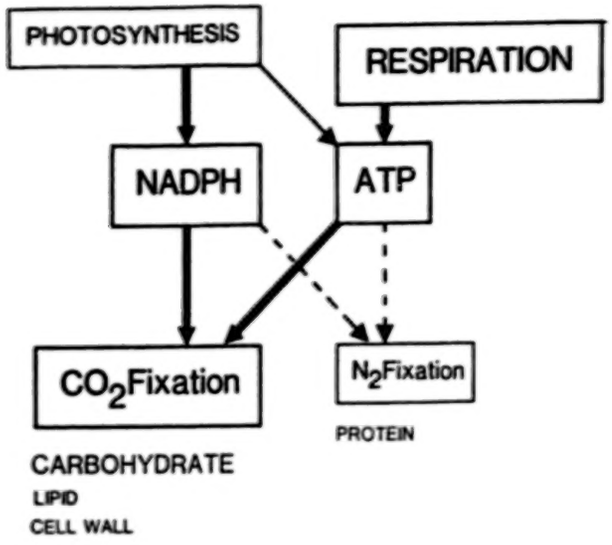

Fig.5 Redirection of photosynthetically derived reductant under stress conditions.

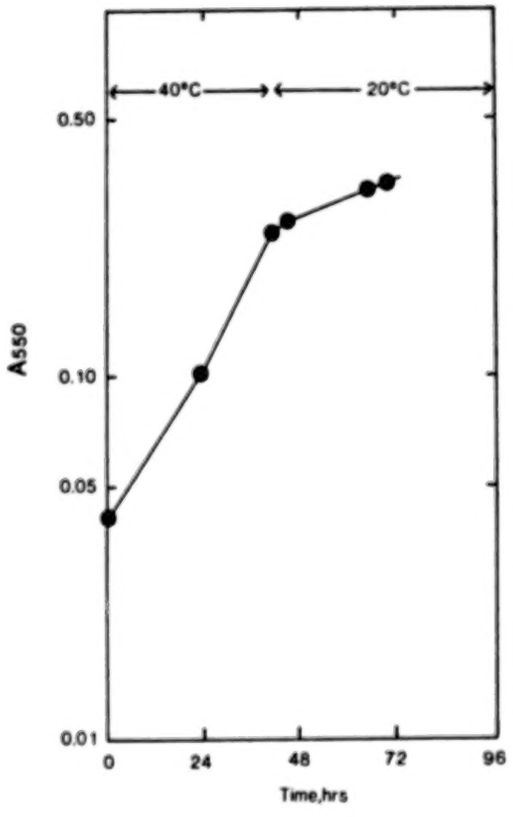

Fig.6 Effect of temperature on the growth of Synechococcus 6311.

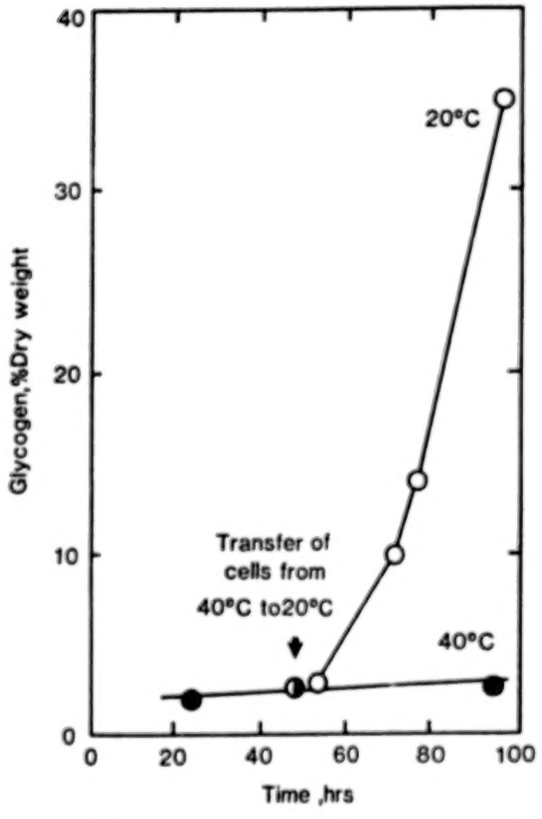

Fig.7 Effect of temperature on the glycogen content of Synechococcus 6311.

TABLE I Average Daily Requirements For Humans

Food Type	Daily Requirement*	
	Grams	%
Protein	330	21
Carbohydrate	825	52
Lipid	429	27
TOTAL	1584	100

Assumptions:

* Body weight of 70kg (150 lbs.)

TABLE II Rates of N_2 Fixation and Photosynthesis During Growth in Airlift Fermenter

	Maximal Rate*	Fermentor Rate
Photosynthetic O_2 evolution (umoles O_2/mg·chlorophyll/hr)	300	72+
N_2 fixation (umoles C_2H_2 reduced/mg·chlorophyll/hr)	25 (air) 46 (N_2)	43#

* direct measurements from diluted samples

+ calculated from total CO_2 plus N_2 fixed (as carbohydrate and protein)

calculated from the total N_2 fixed as protein

TABLE III Factors Affecting The Carbohydrate Content Of Synechococcus 6311

Growth Condition (30°C)	Generation Time (hr.)	Respiration (umoles O_2/mg chl/hr.)	Glycogen (% Dry Wt.)
Control	19.7	2.9	0.9
+0.5M NaCl	24.0	20.0	12.0
+60ppm SeO_4^{2-} (SO_4^{2-} = 14ppm)	32.0	8.0	2.2
Grown at 20°C	33.1	41.3	35.0

Photosynthesis (after 48 hrs) is at control rates in all cases

TABLE IV Effect Of Growth Temperature On Synechococcus 6311

Growth Temp. (°C)	Generation Time (hr.)*	Photosynthesis (umoles O_2/mg chl/hr.) * #	Respiration (umoles O_2/mg chl/hr.) * #	Glycogen (% Dry Wt.)
40	20.1	180.7	3.6	1.7
30	19.7	178.2	2.9	0.9
20	33.1	198.8	41.3	35.0

* Determintions made on 72 hr culture

Assayed at growth temperature (Q_{10} (photosynthesis)=35%, Q_{10} (respiration)=68%, between 30°C & 40°C for 30°C & 40°C grown Cultures)

Synechococcus 6311 /2/, and we have continued to use this organism, in addition to the nitrogen fixing cyanobacterium Nostoc muscorum. Both CO_2 fixation and N_2 fixation utalize the pools of photosynthetic reductant and ATP (fig. 1), and we have investigated environmental facators such as salinity, growth inhibition and temperature effects on the distribution of the reductant between N_2 or CO_2 fixation, to determine the feasibility of using such effects to direct the photosynthate to one particular pool of macromolecule.

RESULTS

Measurements of photosynthetic electron transport (O_2 evolution) and nitrogenase activity (C_2H_2 reduction) in Nostoc muscorum show that only 8% of maximum electron transport ($4e^-$ per O_2 evolved, $2e^-$ per C_2H_2 reduced) is utalized for N_2 fixation (Table II) and one would expect high levels of CO_2 fixation to occur. However, estimations of rates of actual photosynthesis during growth in an airlift fermentor (fig. 2), calculated from the rates of carbohydrate and protein formation, show that the total photosynthetic rates are much lower, probably due to a cut off effect, the attenuation of light by the density of the culture during growth. It is interesting to note that, even under this reduced photosynthetic activity, the rate of N_2 fixation is maximal (Table II). Clearly, N_2 fixation, under these conditions, has priority for photosynthetic reductant, and is probably the limiting factor for growth under these conditions.

SALINITY EFFECTS

Blumwald and Tel-Or have reported the effects of salinity or N_2 fixation (5) which is clearly inhibited during the first 2 days (fig. 3). Under similar conditions, using Synechococcus 6311, we have reported the marked accumulation of glycogen (/2/ & fig. 4). In both cases, salt resulted in a retardation of growth /2,5/ and to a much lesser degree, photosynthesis /2/. Under conditions where growth rate is reduced more than photosynthesis, reductant is directed towards CO_2 fixation rather than N_2 fixation (fig. 5).

FACTORS AFFECTING CELLULAR GLYCOGEN CONTENT

Table III presents the effects of several regimes employed to reduce growth rates, while not affecting photosynthesis. Both NaCl and selenate (a competative inhibitor of sulfur metabolism) reduce growth rates and stimulate glycogen content. The greatest effect is observed, however, when cells are grown at below optimal temperature (20°c).

TEMPERATURE EFFECTS

Growth of Synechococcus 6311 at different temperatures is shown in table IV, and while photosynthesis is relatively unaffected by growth temperature, the generation time is almost doubled and glycogen content increased by a factor of 39 at 20°c.

Although this presents a useful tool for the modification of biomass towards carbohydrate, growth rate (and therefore total biomass production) is slow at 20°c. Therefore, cultures of Synechococcus 6311 were grown at 40°c for two days, the temperature then being reduced to 20°c. Fig. 6 demonstrates the rapid inhibition of growth during cold treatment of an already dense culture of Synechococcus 6311. Analysis of samples taken before and after transferto 20°c, show a marked accumulation of glycogen during the period of growth inhibition (fig. 7).

CONCLUSIONS

Results so far indicate that temperature modification is the most effective tool for the manipulation of the biomass in favor of glycogen. This technique is particularly attractive, since the algae subsystem would only require adjustment of the cooling system, without manipulation of the nutrient supply. Future experimentation will continue along this line of research, using established food compatible systems (e.g. Spirulina).

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N88-12258

SUNLIGHT SUPPLY AND GAS EXCHANGE SYSTEMS IN MICROALGAL BIOREACTOR

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ABSTRACT

The bioreactor with sunlight supply system and gas exchange systems presented here has proved feasible in ground tests and shows much promise for space use as a CELSS device.
Our chief conclusions concerning the specification of total system needed for a life support system for a man in a space station are the following.
(1) Sunlight supply system : compactness and low electrical consumption.
(2) Bioreactor system : high density and growth rate of chlorella.
(3) Gas exchange system : enough for O_2 production and CO_2 assimilation.

INTRODUCTION

The sunlight supply system /1-2/ presented will provide the visible solar radiation necessary for the various activities in the space station, such as cultivation experiments on algae, plants, fishes, birds, and animals room, lighting for modules, and crew sun-bathing. Even natural solar rays reaching the earth's surface contain ultraviolet rays of medium and long wavelengths (UV-B and UV-A) and infrared and heat rays, all of which are harmful to life. On a space station, the most dangerous short-wavelength ultraviolet rays (UB-C), x-rays, and gamma-rays are added, and those cited above are present in markedly higher intensity. It will thus only be after elimination of these harmful rays that spacial solar rays will be utilizable in the space station for activities related to the life sciences. With respect to the Regenerative Life Support System in CELSS, the main function of the microalgal bioreactor with gas exchange system to supply O_2 and assimulate exhaust of CO_2 for a man in a space station, though another would be to produce algal biomass for a human food and medicine: the biomass protein, carbohydrate, and β-carotene would be useful.

CONCEPT AND BASIC DESIGN OF TOTAL SYSTEM

Sunlight Supply System

The basic design of the sunlight supply system is shown in Figure 1 /1/. The solar rays are collected and concentrated by a factor of 10,000 by means of a solar collector assembly composed of many small Fresnel lenses.

Limitation of the admitted spectral band to the visible region (380-770 nm wavelength) is made possible by the chromatic abberation of the individual Fresnel lenses. Chromatic aberration caused by the Fresnel lenses separates the focal points of different wavelengths, so that placing the end of the fiber optic cable at the focus of green light admits mainly the visible spectrum (Figure 2). The solar rays focused by each small lens are fed through the well-polished terminal end of a flexible light-conducting fiber, and are confined within its quartz core for transmission to the required location.

Solar collection is performed by Fresnel lenses assembled in a honeycomb lattice as illustrated in Figure 1. The front face of each is covered by a protector serving also as a filter. The protector is made of quartz, is resistant to scratching, and is sufficiently thick to withstand most impinging meteoroids, debris and deposits that may be encountered. Moreover, in the event of damage to the protector, the affected unit can be cut off and sealed off from the rest of the system, which can continue functioning ing independantly. The space between protector and array

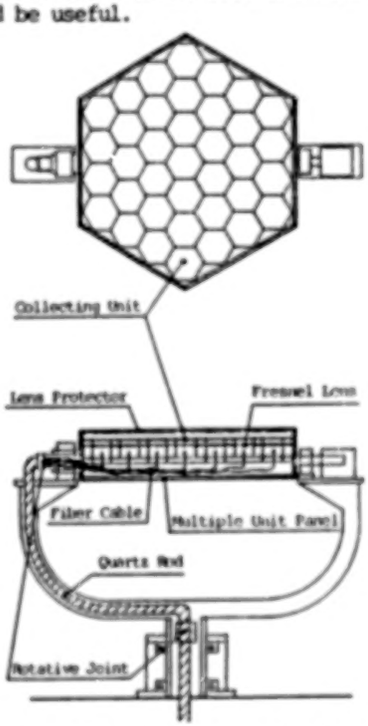
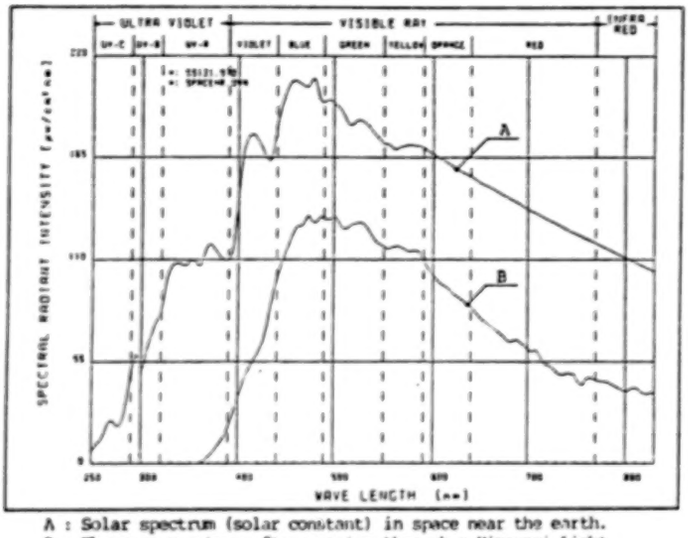


Fig.1 Basic design of solar collector.

independently. The space between protector and array is filled with an inert gas to allow temperature control and to suppress evaporation of the component materials.

Figure 3 is a graph showing the HIMAWARI light collector's visible spectrum transmissivity. As can be seen in Figure 3, almost all ultraviolet radiation is excluded, while infrared heat radiation is cut by about forty percent. Light spectrum power analysis was carried out with a Princeton Applied Research Model 1450 OMA with an 0.5 nm wavelength increment



A : Solar spectrum (solar constant) in space near the earth.
B : The same spectrum after passing through a Himawari Light Collector of 60% transmissivity.

Fig.3 Graph demonstrating the Himawari Light Collector's visible spectrum transmissivity and its blocking of UV and IR wavelengths in space.

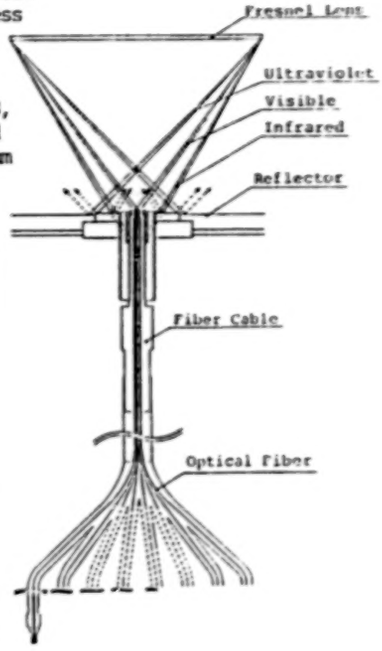


Fig.2 Fresnel lens chromatic aberration light filtration system.

connected to an optical fiber branched off from the main trunk.

Microalgal Bioreactor System

Within the hexagonal bioreactor tank, the tubular fiber-optic light radiators /3/ are arranged vertically in a uniform pattern to provide a high radiative surface per volume of algal suspension (Figures 4 and 5). Light entering the tank through the fiber-optic cables passes into acrylic optical rods (light guides), which form the centers of the light radiators. The cladding of these radiators has a lower refractive index than conventional quartz optical fibers.

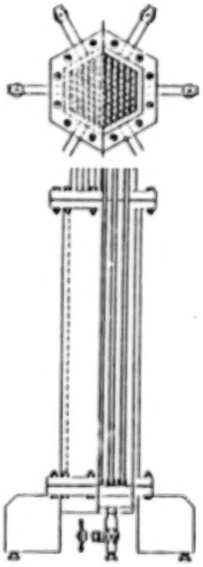


Fig.4 Photoautotrophic bioreactor tank.

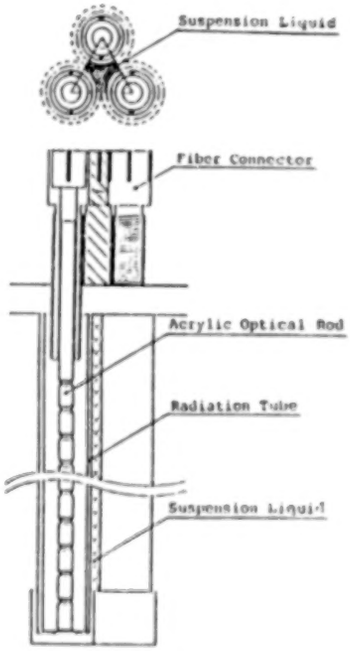
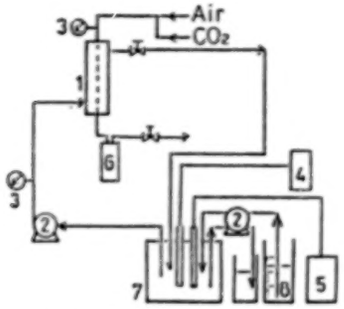


Fig.5 Fiber-optic light radiator detail.

Direct immersion of the acrylic rods in the algal suspension results in little light efflux as well as clogging of the light openings with microalgae. Encasing the rods in air-filled acrylic tubes solves both problems, and also helps scatter the light. The maximum light path through the suspension from luminant surface to algal cell is 1.5 mm, and most cells are illuminated from all sides. Wedge-shaped cuts passing through the cladding and spaced at one-centimeter intervals allow light to escape evenly along the rod length to illuminate the algal suspension (Figure 5).

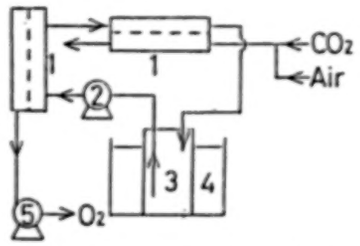
Gas Exchange System

Figure 6 shows a schematic flow diagram of the system used for measurement of carbon dioxide dissolution into the solution from the gas phase. It uses a hollow fiber module made of micro-porous polypropylene membrane, which was supplied by the Mitsubishi Rayon Co., Ltd.. Figure 7 shows the schematic flow diagram of the system used to recover dissolved oxygen from the solution, which uses the same hollow fiber module. The bore of the hollow fiber was kept at reduced pressure so as to increase partial pressure difference of O_2 over the micro-porous membrane.



1.Hollow Fiber Module 2.Pump 3.Pressure Gauge
4.CO_2 Desitometer 5.pH Controller 6.Trap
7.Bioreactor 8.NaOH Solution

Fig.6 Apparatus for measurement of carbon dioxide dissolution using hollow fiber module.



1.Hollow Fiber Module 2.Pump 3.Vessel
4.Constant Temp. Bath 5.Vaccum Pump

Fig.7 Oxygen recovery system.

Theoretical methods. Carbon dioxide dissolution. The bioreactor contained one liter of pure water, which was circulated through the module at a fixed flow rate (Figure 6). The mixture of air and CO_2 was fed into the bore of the hollow fiber and dissolved into the water flowing outside of the fiber. The concentration of CO_2 in the water, C_{AL}, was measured by CO_2 destometer and recorded. Assuming overall mass transfer coefficient, K_L, constant, C_{AL} at any time may be expressed by the following equation:

$$\frac{C_A - C_{AL}}{C_A - C_0} = \exp(-K_L \cdot a \cdot t) \qquad (1)$$

where $C_A = H \cdot P_A$ (2)

P_A, H, and a are partial pressure of CO_2 in the gas mixture, Henry's constant, and specific membrane area, respectively.

Oxygen recovery. Water dispersed with tiny bubbles of air (instead of oxygen) was fed into the module, where it flowed outside of the hollow fiber. The bubbles attached to the surface of the micro-porous membrane and disappeared gradually; they were sucked through the membrane because the pressure of the bore was kept at about 180 mm Hg. Water did not permeate through the membrane.

Characteristic. Figure 8 shows the effects of concentration of CO_2 on K_L at two levels of water flow rate. Figure 9 shows the effects of water flow rate on K_L. The values of K_L shown in figure were analyzed by Wilson's method to obtain liquid phase mass transfer coefficients, K_L, and K_G. The overall mass transfer coefficient, K_L, in this case may be expressed as follows:

$$\frac{1}{K} = \frac{1}{K_L} + \frac{I}{D} + \frac{1}{H \cdot K_G} \qquad (3)$$

$$\frac{1}{K_L} = 1.28 \cdot 10^{-7} \cdot V^{0.6} \qquad (4)$$

where D/I = 0.248 [m/s]

Where I and D are membrane thickness and diffusivity, respectively, of CO_2 in the gas mixture. V is flow rate of water in terms of m^3/s.

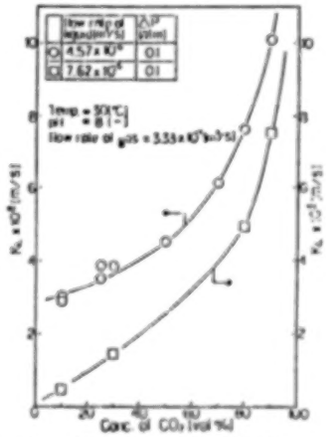


Fig.8 Over-all mass transfer Coefficient K_L heavily depends on the concentration of carbon dioxide in the gas mixture.

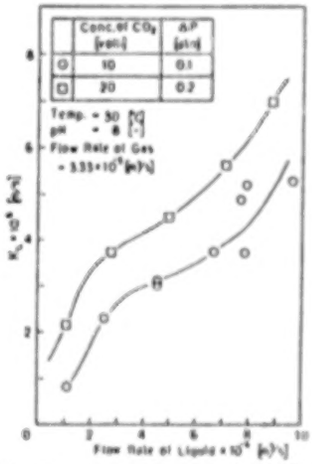


Fig.9 Over-all mass transfer coefficient K_L increases with flow rate of liquid.

EXPERIMENTAL TEST

The bioreactor system consists of the light collection and transmission apparatus, the photoautotrophic bioreactor, and the membrane device for gas exchange (Figure 10). Measurement and control devices were employed for experimentation.

Bioreactor Testing without Gas Exchange System

Apparatus and methods. The light collector, called the "HIMAWARI" /4/, is produced commercially by a Tokyo-based engineering firm, La Foret Engineering and Information Service Co., Ltd.. The optical fibers are specially manufactured to be transparent to the visible spectrum (transmissivity of 96% per ten meters), since those used in the communications industry are transparent to the infrared region. Chlorella was cultivated within the bioreactor in two medium mixtures, one of pH 4.0-4.5 consisting of (mg/L): N, 63.572; P, 227.608; K, 287.308; Na, 4.689; Mg, 394.458; Fe, 0.500; B, 0.500; Mn, 0.500; Cu, 0.200; Mo, 9.784, and one consisting of Mayers-4NA5. Innocula were cultivated for two or three weeks in aerated and illuminated (5% CO_2 air at 30 L/h and 10,000 lx) glass bottles before being suspended in the bioreactor medium.

Results. Figure 11 shows the effect of temperature of chlorella solution upon the growth rate of chlorella. Although such temperatures are commonly much higher, the optimum growth temperature for chlorella occurred at 24-26°C for this bioreactor at the gas mixture given earlier, perhaps because of the unique nature of visible spectrum light. Figure 12 shows that the rate of chlorella density change increases with density but levels off due to attenuation of the incoming light. Eventually, the rate of density change is adversely affected by increasing the density and an optimum is reached. To control the density constantly is thus found to be an important operation for minimum energy loss. Figure 13 shows that the rate of chlorella density change changes linearly with respect to the visible light transmission energy. By approximating this linear relationship, higher value data points were plotted.

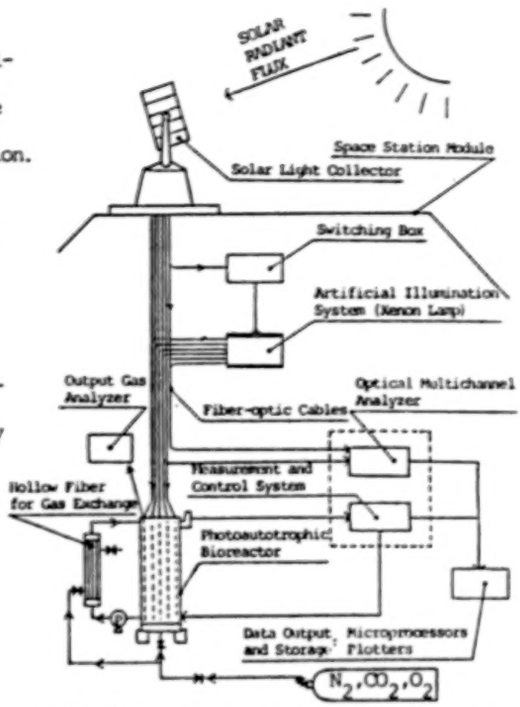


Fig.10 Bioreactor system schematic diagram.

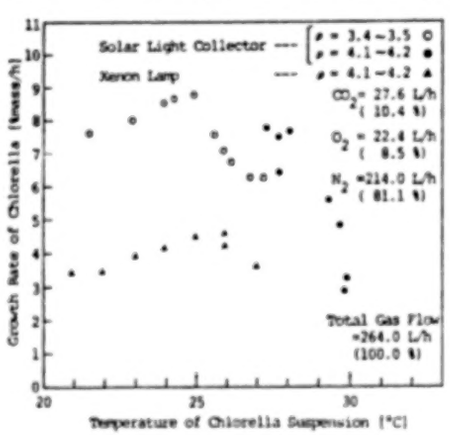

Solar Light Collector
Xenon Lamp
CO_2 = 27.6 L/h (10.4 %)
O_2 = 22.4 L/h (8.5 %)
N_2 =214.0 L/h (81.1 %)
Total Gas Flow =264.0 L/h (100.0 %)
Growth Rate of Chlorella (Mass/h)
Temperature of Chlorella Suspension [°C]

Fig.11 Effect of temperature of chlorella growth rate.

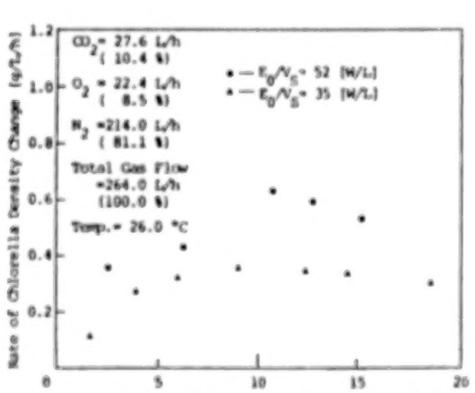

CO_2 = 27.6 L/h (10.4 %)
O_2 = 22.4 L/h (8.5 %)
N_2 =214.0 L/h (81.1 %)
Total Gas Flow =264.0 L/h (100.0 %)
Temp.= 26.0 °C
Rate of Chlorella Density Change [g/L/h]
Density of Chlorella in Basic Solution : ρ [g/L]

Fig.12 Relationship between rate of chlorella density change and density of chlorella in basic solution.

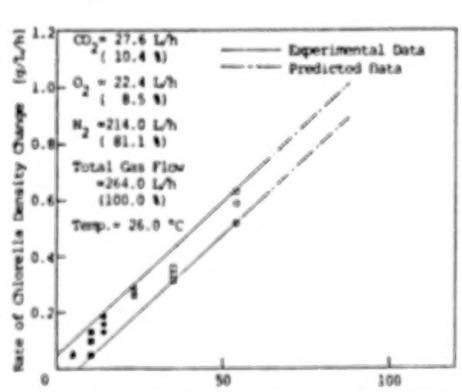

Experimental Data
Predicted Data
CO_2 = 27.6 L/h (10.4 %)
O_2 = 22.4 L/h (8.5 %)
N_2 =214.0 L/h (81.1 %)
Total Gas Flow =264.0 L/h (100.0 %)
Temp.= 26.0 °C
Rate of Chlorella Density Change [g/L/h]
Visible Ray Transmission Energy per Liter [W/L]

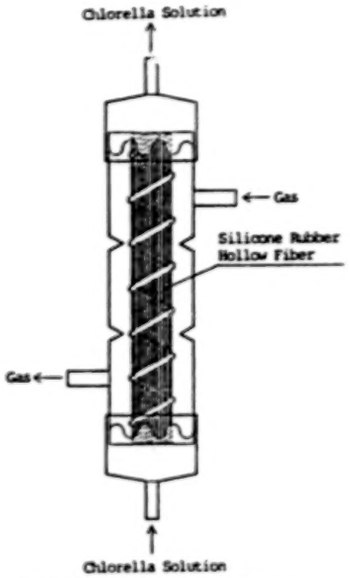
Fig.13 Relationship between rate of chlorella density change and visible ray transmission energy per liter.

Bioreactor Testing with Gas Exchange System

Apparatus and methods. In this testing membrane for gas exchange, the silicone-rubber hollow fiber module was used because this one did not show loadings and a change with passage of operation time. The value of silicone K_L ($8.4*10^{-6}$ [m/s]) is higher than polypropylene one. The membrane unit case is made of glass and can be autoclaved. This membrabe device was designed by La Foret Engineering, and manufactured by Fuji-Systems Co.. Its specifications are shown in Figure 14.

The operation conditions of gas exchange system are shown in Table 1. Input power levels of the Xenon lamp were 9W, 5W, and 3W in repeated cycles. The incident light intensity in a low earth orbit typically consists of alternating periods of thirty minutes of darkness and sixty minutes of light. Xenon lamps are used as an artificial, controllable source of illumination because of the similarity of their visible spectrum to that of the sun. Xenon lamps coupled with parabolic mirrors to produce a parallel flux can use the same chromatic aberration filtering and fiber-optic ducting as that of sunlight. Because the lamps are separated from the bioreactor by the fiber-optic cables, waste heat is not a concern.

The chlorella solution recycled through the bioreactor and membrane device for gas exchange. The levels of DO and DCO_2 were controlled through the pressure and flow rates of the CO_2 and N_2 supplies. In this test, the level of O_2 production could not be analyzed directly. Therefore, the level of O_2 production and chlorella production were calculated by the rate of DCO_2 change.



Material	Silicone Rubber
Inner Diameter [mm]	0.60
External Diameter [mm]	0.90
Wall Thickness [mm]	0.15
Effective Membrane Area [m^2]	0.082

Fig.14 Membrane device for gas exchange.

Table 1 Comparision between Silicone and Polypropylene.

	Specification		Operating condition							Permeability 1)	
Gas exchange membrane	K_L [$*10^{-6}$m/s]	Effective membrane area [m^2]	Volume of solution [L]	pH [-]	ΔP [atm]	Flow rate of liquid [$*10^{-5}$m /s]	Flow rate of gas [$*10^{-5}$m /s]	Temp. [°C]	Conc. of CO_2 [vol%]	O_2	CO_2
Silicone	8.4	0.082	3.1	4.5 ~6.5	0.20	8.33	8.33	25	10	1.57 $*10^6$	8.04 $*10^6$
Polypropylene	0.9 ~6.9	0.500	1.0	8.0	0.10 ~0.20	3.33	0.10 ~0.90	30	10 ~20	2.90 $*10^3$	9.91 $*10^3$

1) membrane thickness 25μ conversion, 25°C, $cm^3/m^2 \cdot atm \cdot 24h$

Results. Figure 15 shows Orbit cycle lights alternation's effect on DCO_2 and DO concentration. During the light periods DO concentration increased while that of DCO_2 decreased. The opposite occurred during the dark periods. The photosynthetic reaction was demonstrated from Figure 15. During each illumination cycle, the DO and DCO_2 concentration pattern was repeated. Figure 16 shows the relationship between visible ray transmission energy and rate of O_2 production. The performance of the membrane gas exchanger was constant through the long duration of the experiment.

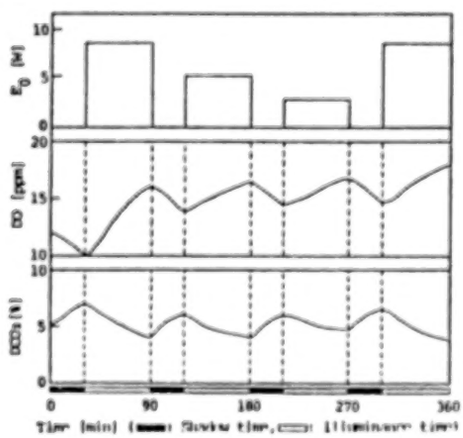


Fig.15 Orbit cycle alternating light's effect on DCO_2 and DO concentration.

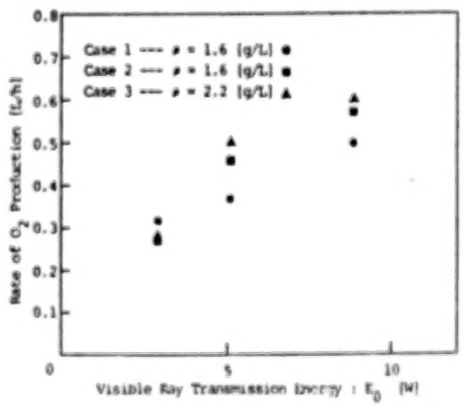


Fig.16 Relationship between rate of O_2 production and visible ray transmission energy.

CONCLUSIONS

The bioreactor with sunlight supply system and gas exchange systems presented here has proved feasible in ground tests and shows much promise for space use as a CELSS device. Our chief conclusions concerning the specification of total system needed for a life support system for a man in a space station are the following (Table 2).

(1) Sunlight supply system : area of solar ray receiving lens : 6.29-9.37 m^2, electrical power consumption : 37-56 W.

(2) Bioreactor system : volume of chlorella solution : 50 L, radiative surface per liter : 0.58 m^2/L, size of bioreactor tank : 0.4 mø x 1.0 m.

(3) Gas exchange system : effective membrane area of silicone-rubber hollow module : 1.33 m^2.

Chlorella photosynthetic efficiency is 7%, which is higher than that one of high plant (0.1-1.0%). Microalgal bioreactor system will be best system for a life support system in a space station.

Table 2 Required performance and simulated specification of total system.

Case	I		II		III		Source
Light condition	Illuminance time: 60 [min]	Shadow time: 30 [min]	Illuminance time: 60 [min]	Shadow time: 30 [min]	Illuminance time: 60 [min]	Shadow time: 30 [min]	
Light source	Solar ray E_0	Xenon lamp E_0*100%	Solar ray E_0	-	Solar ray E_0	Xenon lamp E_0*10%	
Total mass of O_2 produced per day in tank [g/day] [1,2]	686*1.1=754.6		686*1.1=754.6		686*1.1=754.6		Ref.5
Average mass of O_2 produced per hour during light time [g/h]	31.4	31.4	47.2	-	44.9	4.49	
Chlorella biomass produced per hour [g/h]	29.5	29.5	44.2	-	42.1	4.21	
Rate of chlorella density change [g/L/h]	0.59	0.59	0.88	-	0.84	0.08	
Volume of chlorella solution [L]	50		50		50		
Radiative surface per liter [m^2/L]	0.58		0.58		0.58		Ref.2
Visible ray transmission energy per liter needed in tank [W/L]	49 [a]	49 [a]	73 [b]	-	69 [b]	6.9 [a]	Fig.13
Temperature range of solution [°C]	25±1		25±1		25±1		Fig.11
Density range of chlorella in solution [g/L]	about 10 [a]		about 15 [b]		about 15 [b]		Fig.12
Chlorella photosynthetic efficiency : Xe [%]	7		7		7		formula(5)
HIMAWARI transmission efficiency : η [%]	60	-	60	-	60	-	Ref.1
Area of solar ray receiving lens : S_L [m^2]	6.29	-	9.37	-	8.85	-	formula(6)
Visible ray transmission energy : E_0 [W]	2450	2450	3650	-	3450	345	formula(6)
Electrical power consumption [4,5] [W]	37	24500	56	-	53	3450	Ref.6
Gas exchange module	Silicone-rubber hollow fiber						Fig.14
Effective membrane area [m^2]	Max. 0.082*50[L]/3.1[L] = 1.33						Fig.14
Size of module [mm]	145ø * 450						

a) experimental data b) predicated data

1) Average mass of O_2 needed per day for a man in a space station is 686 g /5/.
2) The rate of O_2 consumption as chlorella respitation (basal metabolism) is 10% of photosynthetic biomass.
3) Definition of the formula

$$Xe = \frac{\Delta Ch \cdot E_{ch}}{E_0 \cdot 860 [cal/W]} \quad (5) \qquad , E_0 = A \cdot k \cdot S_L \cdot \eta \quad (6)$$

ΔCh : change in total mass of chlorella [g] E_{ch} : energy per unit biomass chlorella : 5.6[kcal/g]
A : Solar constant value : 1.353[W/m^2] k : visible spectrum energy fraction : 48[%]

4) The electrical power consumption of HIMAWARI per one square meter of lens is 5.93[W/m^2] /6/.
5) The rate of Xenon lamp electrical power change for visible ray transmission energy is 10[%].

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N88-12259

A REVIEW OF RECENT ACTIVITIES IN THE NASA CELSS PROGRAM

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ABSTRACT

A CELSS (Controlled Ecological Life Support System) is a device that utilizes photosynthetic organisms and light energy to regenerate waste materials into oxygen and food for a crew in space. The results of theoretical and practical studies conducted by investigators within the CELSS program suggest that a bioregenerative life support system can be a useful and effective method of regenerating consumable materials for crew sustenance. Experimental data suggests that the operation of a CELSS in space will be practical if plants can be made to behave predictably in the space environment. Much of the work currently conducted within the CELSS program centers on the biological components of the CELSS system. The work is particularly directed at ways of achieving high efficiency and long term stability of all components of the system. Included are explorations of the conversion of non-edible cellulose to edible materials, nitrogen fixation by biological and chemical methods, and methods of waste processing. It is the intent of the presentation to provide a description of the extent to which a bioregenerative life support system can meet the constraints of the space environment, and to assess the degree to which system efficiency and stability can be increased during the next decade.

INTRODUCTION

The Controlled Ecological Life Support System (CELSS) program is a research effort of the National Aeronautics and Space Administration (NASA) that has the goal of providing the research and technology base required to develop a bioregenerative life support system for use in extraterrestrial environments. Such environments include orbiting space stations, the lunar surface, transits to the outer planets, and bases on Mars and the asteroids. Recently, the National Commission on Space released its report /1/ which contains an exhilarating plan for American space exploration during the next half-century. The Commission recognized seven critical technologies that are essential for achieving their proposed space efforts; one of the seven is the development of long duration "closed-ecosystems". The approach of the CELSS program to fulfill this need is to explore first the development of a practical ground-based bioregenerative life support system, usable in the relatively near term, but to maintain close and active involvement with the planners who are developing concepts for larger, more complex systems /2/ suitable for colonies on the Moon or other planets /3,4/.

Bioregeneration is a feasible method of life support for those missions with large crews, or that are long-term and that cannot be resupplied easily or economically /5/. The CELSS concept is based on the natural processes of recycling that occur in the terrestrial environment. It is assumed that by artificially creating similar cycles of materials within small, closed systems, a usable ecosystem can be produced which is capable of supporting a crew. However, a CELSS is not directly analogous to the terrestrial ecosystem because its volume, and the sizes of its materials reservoirs are minute, and the rates of material cycling are much faster /6/. Such characteristics dictate that the system be highly engineered and controlled.

A very large amount of scientific investigation and technological development will be required before a CELSS can be built for use in space. However, the directions that investigations must follow are reasonably clear, and such work is proceeding at laboratories around the world. The CELSS program has recently begun to explore the problems associated with scaling-up laboratory studies to sizes appropriate for the support of one person /7,8/. These larger scale investigations will utilize data generated in continuing laboratory studies, but they will also allow assessment of practical problems of life support. This paper will outline current views of critical scientific issues and will summarize plans for investigating the problems associated with large human-sized CELSS facilities. This COSPAR Workshop also includes expositions of recent data and concepts by investigators of the NASA CELSS program, on the growth of higher plants for CELSS (presented by F. Salisbury and by T.

Tibbitts); on the use of algae in a CELSS (presented by Packer, by Radmer, and by Smernoff), and on food processing problems (presented by Karel).

RESEARCH ACTIVITIES AND DRIVERS

Many of the basic CELSS science investigations deal with the transformation of materials. The areas of fundamental research are guided by the need to understand the effect of environmental parameters on materials transformations. Specific examples are the effect of atmospheric CO_2 concentration on the growth rate of wheat plants (the transformation of CO_2 to wheat kernels), or the effect of temperature on the conversion of waste materials to CO_2, nitrogen compounds, and water (transformation of organic materials to CO_2 and water).

CELSS basic science can be considered as falling into distinct research areas which require significantly different scientific approaches. These are:

- Ground based research
- Flight investigations

It is assumed that ground experimentation will precede all flight experiments, and that flight experiments will seek specifically to address only questions concerning the effects of the space environment: principally gravity forces different from terrestrial and an altered radiation environment. Both ground-based and flight research must accomodate investigations that use two different approaches:

- Single variable analysis
- System analysis

Single variable analysis involves the experiments in which all variables except one are held constant and response to the remaining variable is examined. An example is the exploration of plant growth as a function of light intensity, or temperature, or CO_2 concentration.

System analysis explores the coupling effects of variables, and will be significant not only for understanding the operation of the complete mechanical operation of a CELSS, but also for understanding biological activities. For example, the rate of CO_2 uptake by plants is coupled to temperature, light intensity, CO_2 concentration, growth phase, humidity, day length, nutrient composition, and other variables. System analysis is important to all CELSS science because of the need to maintain stable operation of all systems and to maximize efficiency.

It is frequently observed that system functions, particularly complex ones in which all behaviors are not well-defined (*e.g* organisms) exhibit behaviors that are not predictable *a priori* /9/. While such behaviors may present no practical difficulties to the operation of a CELSS, it is prudent to demand that such systems be observed carefully for indications of instability /10/, and that methods for overcoming possible instabilities be evaluated.

PRACTICAL ISSUES

The fact that the CELSS program can identify an end-item — a CELSS operating in an extraterrestrial environment — provides a focus for the program's research, but also allows the introduction of constraints which effectively narrow the scope of the scientific investigations. The primary constraints reflect the fact that, no matter where the operating site of a CELSS is found (in space, or on the lunar or martian surfaces), the system must be constructed to function within the mass, volume and power limitations of space flight.

These fundamental constraints require the use of efficiency of operation as a primary measure of the practicality of components. As a consequence, certain of the applied science directions to be followed can be identified. For example, even crude evaluations of CELSS efficiency allow placement of subsystems for animal food production and for chemical synthesis of food at lower priority levels, compared to subsystems for higher plant or algae growth.

Another significant influence on the direction of scientific studies is the sequence that will be followed in the development of a CELSS, from laboratory investigations through ground-based system demonstrations, flight experiments, man-tested facilities, to the construction and flight of a CELSS module for use in space. At the present time this sequence assumes that after investigations of scale-up, and preliminary component integration, a manned test facility should be constructed. This would be followed by a human-scale, functional mock-up, followed in turn by a module for use in space. Concurrently with ground-based developments, experiments conducted in space will be devised to answer questions about the behavior of components and small systems in the space environment.

Development of a fully operable CELSS useful in space is the ultimate goal of the CELSS program, however, it is very likely that technology that is fully developed at the component level may be found useful for flight before a CELSS is completed. Examples are devices for the growth of salad plants to supplement the diet of a space station crew, or the use of a super-critical water reactor for waste disposal or air regeneration in space station.

CRITICAL SCIENTIFIC AND TECHNOLOGICAL RESEARCH ISSUES

Several areas of investigation fall into the category of critical issues, primarily because solutions to these problems are essential for consideration of a CELSS as a usable system, and because current information is insufficient to evaluate them properly. Examples of such critical issues that require investigation are:

Efficiency: minimizing the mass, power and volume required for the growth of organisms to provide complete life support capabilities within the constraints of a mission;

Stability: understanding the behavior of essential organisms to allow predictable reliability of system operation over time periods of 2 to 10 years;

Space radiation: evaluating and minimizing its effects on growing organisms;

Gravity: understanding its effects on growing organisms sufficiently to allow the operation of a CELSS, with the possibility of reasonable centrifuge assist, within the gravity environment of the mission.

Human Labor Requirements: reduction of human labor requirements to a minimum through the use of automation and robotics, and simplification of man-machine interactions through the use of expert systems and mathematical models predictive of future system states.

Each of these issues is discussed in more detail below with the intention of identifying areas which require technology development.

Efficiency

The projected efficiency of a CELSS in operation in space will depend on many factors. For example: the mass of the equipment required to operate the system; the mass of water required to operate the biological subsystems; the volume that the system occupies, and the extent to which volume can be reduced by packing; the extent to which materials can be recycled, thus reducing resupply or stowage requirements; the power demands of the system, and the extent to which electrical power requirements can be reduced by introducing alternate lighting systems.

One of the elements in a CELSS to which the system is most sensitive is the food production subsystem. As an example, current estimates /11/ suggest that 18 to 20 m^2 of growing wheat, when harvested and planted daily, will produce enough edible biomass to supply 2800 calories per day for human consumption. If the area required could be reduced to 15 m^2, a reduction of 17 to 25%, the mass of plant growth equipment, water, lighting equipment including solar panel equipment, and heat rejection equipment, could also be reduced proportionately. Alternatively, the amount of food available for crew use could be increased by the same amount. It is thus of great interest to explore various means to increase food production efficiency.

Efficiency increases in food production may be sought in many areas, including increasing the ratio of edible to non-edible biomass of plants; increasing the yield of individual plants; selecting highly productive crop plants; decreasing the time required for plant maturation; and converting inedible biomass to edible materials. Increases in photosynthetic efficiency may be useful, although the gains in system efficiency would be less marked than for other improvements. It should be noted that comments made by scientists from the Soviet Union suggest that the productivity of wheat can be increased, through selection of cultivars and identification of optimal environmental conditions, to a level equivalent to use of only 6 to 7 m^2 /12/.

While efficiency is currently measured in laboratory settings, using small plots, or small numbers of plants, there is a likelihood that much larger planting areas may be required in an operating CELSS. It is of considerable interest, therefore, that an increased scale of operation be explored, and that larger, integrated systems be tested.

Stability

The concept of stability has both simple and complex aspects. Applied to a working system, the concept implies that the system will function, with proper maintenance, for as long as is necessary. Mechanical systems generally have the advantage that they can be turned on or off as appropriate; however, some mechanical systems must

operate continuously and indefinitely. An example of the latter might be the cooling system of a nuclear power plant. To address the reality that a failure might occur requires introduction either of redundancy or of some reliable measure of failure rate that can be factored into the estimate of reliability that is required.

Biological systems are also referred to as "stable", but the meaning is slightly different because it includes the knowledge that a biological system (perhaps a single organism) will age and will enter into a sequence of "stages" in its life cycle. Groups of organisms (fields of wheat, forests) are recognized as stable within well defined limits (*e.g.* until maturity). From the perspective of ecology, stability is a term that may be applied to a group of very diverse organisms, ranging from microbes to mammals, that are defined as members of an ecosystem, but again with the recognition that the term also includes the concept of succession of species, variations in populations within limits, and possibly as describing stages within evolutionary sequences.

Stability in a CELSS refers to the function of the system. The system is a complex one, and includes mechanical, chemical, physical, and biological components. By far, the components of such a system that are potentially the most unpredictable, unreliable and unstable are the biological ones. Recognition of this issue calls for thorough consideration of CELSS stability as a major research issue.

Of particular significance is the reliable and predictable growth of crops. A plant operates as a system that, over time, develops through what is usually seen as a sequence of phases, but what is, in fact, a continuum of many activities. Many of the activities can be observed to develop function, sustain the function for a period of time, and then cease the function. In higher plants the key to the sequence of these functions seems to lie in the major process of reproduction. Each of the processes are directly or indirectly affected by the environmental conditions.

The purpose of the CELSS research program is not to understand in detail the individual functions of a plant; rather, it is to assure that the functions occur in a reliable and predictable way. The hypothesis that develops from such considerations is that by maintaining all environmental conditions at known and appropriate levels at all times, the use of plants as the prime elements of a CELSS can allow stable, predictable and reliable system operation.

The basis for this hypothesis is that each of the functions of a plant can be considered to operate as a non-biological chemical reaction: when reactants are supplied at a constant rate, and products are removed at a constant rate, the reaction will proceed in a predictable fashion.

Mating biological systems, containing their own innate control mechanisms (and that can be manipulated with generally unknown consequences), with physical and chemical systems (that can be manipulated with known consequences) will be difficult, but not impossible. It is expected that understanding the extent of the problem, which is one of the goals of CELSS science, will permit practical solutions to potential problems.

Space radiation and Gravity

The effect of continuous, accumulating, and increased levels of radiation on seed intended for establishing crops is presently unknown. It can be anticipated that extensive investigations must be done to establish that seed grown in one crop is suitable for planting a second. The doses of radiation will vary significantly with the mission; a low Earth orbit space station CELSS will be subject to less damaging radiation than will one in geosynchronous orbit, or a CELSS on the lunar surface. Protection of growing plants from radiation is possible, but the increased mass necessary for radiation absorbers must be weighed against the detrimental effects. Initially it is likely that planting seed for a CELSS will be grown on Earth and transported to space in radiation absorbing containers.

The combined effects of radiation and micro-gravity has been suggested for study in a group of organisms, and plants may offer a good study vehicle. The effect of lowered gravity on plant and crop growth is perhaps the most important CELSS-in-space issue that should be studied in the next decade. The issues involved are complex and will be studied in concert with wider investigations of gravitational biology and with NASA's Space Biology program. The interactive influence of radiation must also be explored.

Human Labor Requirements

The primary human labor requirement in a CELSS will be associated with plant cultivation. Most of the activities, however, can be automated, including planting and harvesting of crops. Food processing and preparation are also activities that can be extensively automated. In some cases, automation will be relatively easily achieved; in other cases, considerable data analysis will be needed, as will the introduction of robotic mechanisms. These issues are under study within the CELSS program /13/.

APPROACH

Scientific investigations will continue to focus on methods of increasing the efficiency of higher plant growth. For example, new varieties of crops that mature much more rapidly will be tested; lighting and temperature regimes that promote growth rates will be explored; and environmental regimes that enhance growth will be studied under well-controlled conditions. Waste management and food processing investigations will become of greater importance as the CELSS research program continues.

The NASA CELSS program has recently begun to develop what it terms a Breadboard Project. The project is NASA's initial approach to assessing problems associated with large bioregenerative life support systems. The CELSS Breadboard Project will provide hardware, systems, and techniques for the production of biomass and oxygen, the preparation of food, and the processing of waste in a controlled recycling system. This project will fabricate, test, and operate a breadboard facility to accomplish a proof-of-concept evaluation. It will characterize system operations and mass and energy budgets.

The project will center around a modified steel chamber, 3.6 m in diameter by 6.7 m tall (volume: approximately 68 m^3) for the growth of plants. The chamber will be placed in operation in late 1986 and will initially study the growth of wheat with the intention of determining how efficiencies obtained in the laboratory can be duplicated on a larger scale. The chamber will provide a unique facility for integrating and testing technologies, such as hydroponic plant culture systems, food harvesting and processing equipment, atmospheric and water regeneration techniques, and lighting systems.

The effort is proceeding under the guidance of a number of CELSS scientists, expert in the fields of food production, food processing, and waste management. As the project develops, it will approach regeneration by closure of the chamber to the entrance and exit of materials, and by increasingly efficient recycling materials. The experience gained in the development and use of the facility will factor into the design of a manned verification unit and eventually into flight equipment.

This combined approach, including research and breadboard tests, will assure that technical questions raised over the course of the program will have a proper scientific base in their answers.

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N88-12260

A Modular BLSS Simulation Model

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ABSTRACT

The coordination of material flows in Earth's biosphere is largely made possible by the buffering effect of huge material reservoirs. Without similarly-sized buffers, a bioregenerative life support system (BLSS) for extraterrestrial use will be faced with coordination problems more acute than those in any ecosystem found on earth. A related problem in BLSS design is providing an interface between the various life-support processors, one that will allow for their coordination while still allowing for system expansion. Here we present a modular model of a BLSS that interfaces system processors only with the material storage reservoirs, allowing those reservoirs to act as the principal buffers in the system and thus minimizing difficulties with processor coordination. The modular nature of the model allows independent development of the detailed submodels that exist within the model framework. Using this model, BLSS dynamics were investigated under normal conditions and under various failure modes. Partial and complete failures of various components, such as the waste processor or the plants themselves, drive transient responses in the model system, allowing us to examine the effectiveness of the system reservoirs as buffers. The results from simulations of this sort will help to determine control strategies and BLSS design requirements. An evolved version of this model could be used as an interactive control aid in a future BLSS.

INTRODUCTION

A life support system that uses biological processes to recycle food, air and water holds the ultimate promise of enabling the human race to successfully become established outside the Earth's biosphere. Such a system will also provide an economic advantage for a variety of space missions contemplated for the immediate future /1/. In order to fulfill its promise, a bioregenerative life support system (BLSS) must be safe, reliable, and flexible. Building a BLSS with these qualities will require a detailed knowledge of such systems, so a predictive tool that will allow a myriad of investigations into BLSS design will be useful. In this paper we will present a model that can be used to investigate the workings of a BLSS.

The many problems faced when designing a BLSS have been explained before in this journal /2/, so we will not dwell on them. It should be noted, however, that there is an immediate economic pressure to make a BLSS as small as possible, so any near-term BLSS will be considerably different from the life support system that we are all most familiar with, the Earth's biosphere. Thus we will abandon the luxury of the large buffers (e.g., the oceans and the atmosphere) that play such a significant role in maintaining the Earth as a living planet. This scale change will make it necessary to know as much about the BLSS as a whole as about its component parts.

Other workers have previously used models to study bioregenerative life support systems /3, 4, 5/. These models were often limited to a specific system design, or were aimed specifically at understanding the nature of the control strategies available in a BLSS. Our aim here is to establish an approach to BLSS modelling that can grow along with the changing concepts of BLSS design. We would like to lay a modelling foundation that could be as applicable to an Earth-orbit BLSS as it would be to a system found in a Lunar base. Hence, the model we propose should be viewed as a fluid, hierarchical structure, where changes at one level do not necessarily involve changes at other levels in the model.

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THE MODEL

Components of a Bioregenerative Life Support System

A BLSS can be said to consist of two basic elements: processors (also know as flow elements or transducers) and storages /5/. The processors change the state of materials in the system, and move them from one storage area to another. In the same way that an earth ecosystem is composed of autotrophic and heterotrophic components /6/, a BLSS must contain processors that fix simple inorganic materials into complex substances that are then broken down by the processors that derive energy from them. The storages in a BLSS are equivalent to the vast reservoirs of inorganic materials found on Earth, but because a BLSS will have only a limited amount of reservoir capacity in the system, the level of control needed over the storages in a BLSS will be much greater.

To begin our modelling of a BLSS we defined a system that is made up of five processors and eight storage reservoirs. Only three of the processors have easy biological equivalents in an Earth ecosystem, the Crew and the Waste Processor represent heterotrophic components while the Plants in the system are autotrophs. The Gas Separator(s) and the Nutrient Mixer are simply a means to maintain a greater amount of control over the reservoirs in the system. The storage elements in the system are either mixed storages (the Crew and PGM Atmospheres, the Food Storage, the Waste Storage, and the Nutrient Solution Reservoir) or storages for pure compounds (O_2, CO_2, H_2O, and HNO_3). These particular reservoirs were chosen to represent the storage types that would most likely be found in an operable BLSS, with the constraint that our model would initially only track the Carbon, Hydrogen, Oxygen, and Nitrogen in the system.

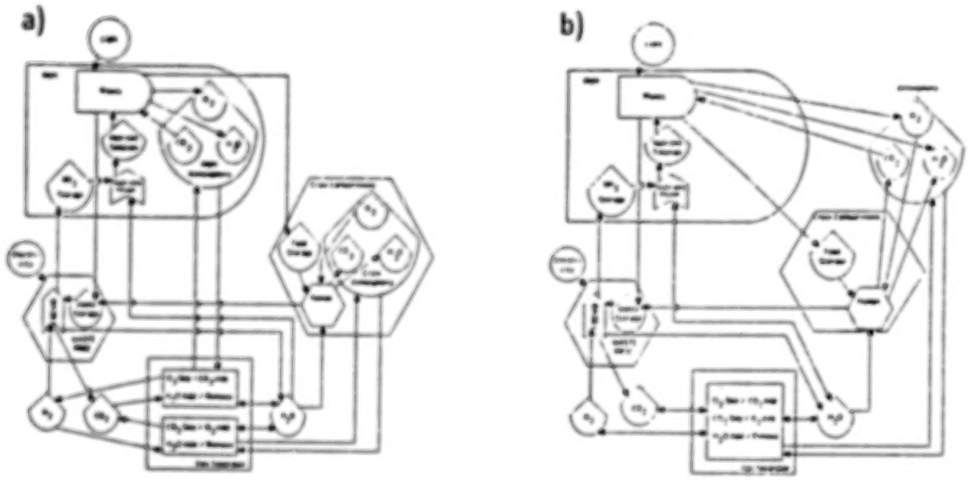

Fig. 1. Two BLSS model structures. In (a) there are two separate atmospheres for the PGM and the crew compartment. In (b) the plants and the people share a common atmosphere.

Structure of the Model

The principal philosophy that guided the structure of the BLSS presented here is that the system should be modular: i.e., each processor subsystem within the model should be able to work (and be modelled) independently of the others. The corollary condition was therefore established that each processor should interface only with storage reservoirs, and not with other processors. Our adaptation of this philosophy is owed, in part, to conversations held with Columbano /7/ at Ames Research Center.

Though the model is intended to represent the flows and transformations that are seen in a BLSS at the macroscopic level, all such transformations were calculated at the atomic level. Only through this means were we able to maintain the high degree of precision in our calculations (on the order of 10^{-9}) needed to conserve the elemental masses represented in the model. Our error level is far below the expected leakage rate of a real bioregenerative system in space. The derivation of the stoichiometry associated with each of the processes in the model is not discussed here, but is given in a companion paper /8/.

The basic structure of two of the BLSS's we studied are shown in Figure 1. The system elements in the figure are portrayed in the energy language of Odum /9/. The BLSS Simulation Model was implemented as a Pascal program running on a VAX 11-785 computer at Ames Research Center. Each of the processors in the system was implemented as a separate Pascal procedure, allowing us to maintain the system's modular design within the program itself. As a result, modifications to the system structure are very easy to make without changing the processors themselves (compare Figure 1a with 1b). A brief description of each of the model BLSS components is given below, along with notes on how they were implemented.

The plants. These processors are found inside of the Plant Growth Module (PGM) shown in Figure 1. For the first iteration of our model we ignored Biblical strictures and postulated that the crew *can* live on bread alone, so the plants grown are wheat plants. These wheat plants interact with the PGM Atmosphere, where they take up or release CO_2 and O_2, depending on the lighting conditions, and where they release H_2O through transpiration. The plants also take up nutrient solution from the Nutrient Solution Reservoir. At harvest time, the edible portion of the wheat is moved to the Food Storage, while the inedible portion of the wheat and the excess edible portion, if any, is sent to the Waste Storage.

The growth of wheat is modelled after data provided to us by Salisbury and Bugbee /10/. Because we did not consider temperature variations, wheat growth was postulated to be dependent only on the lighting conditions, and on the concentrations of CO_2 and NO_3 in the PGM atmosphere. The model for wheat growth is given by the following three equations:

$$\Delta\text{growth} = \text{inedible mass} \times r \times \frac{V_{CO_2} \times [CO_2]}{K_{CO_2} + [CO_2]} \times \frac{V_{NO_3} \times [NO_3]}{K_{NO_3} + [NO_3]} \times \left(1 - \frac{\text{total mass}}{\text{maximum mass}}\right) \quad (1)$$

$$\text{edible mass}_{t+1} = \text{edible mass}_t + \text{allocation} \times \Delta\text{growth} \quad (2)$$

$$\text{inedible mass}_{t+1} = \text{inedible mass}_t + (1 - \text{allocation}) \times \Delta\text{growth} \quad (3)$$

CO_2 and NO_3 concentrations are related to the total growth rate by the use of Michaelis-Menten kinetics coupled to a logistic growth formulation, using the intrinsic growth rate, r, and a cultivar-specific target size for a single wheat plant, **maximum mass** (equation (1)). Furthermore, the net growth of each wheat plant derives only from the inedible portion of the plant (leaves, stalks, etc.). Edible portions are assumed to be able to offset their own respiration by photosynthesis. After a preset time the edible and inedible portions of the plant each receive a constant allocation of the production from the inedible mass (equations (2) and (3)). Before that time is reached only the inedible portion of the plant grows.

These equations can represent the growth of a single wheat plant or of a batch of identical wheat plants. In the model, plant growth is tracked on a per-plant basis. Plants start out from seeds taken from the Food Storage and are allowed to "germinate" (convert from edible to inedible mass proportions) before being planted in the PGM. The growing period we used for this paper lasted the 55 days from transplanting into the PGM until the harvest of the mature crop.

No doubt some fallacies exist within this model of the plants, but using similar conditions to those reported by Salisbury and Bugbee /10/ the model duplicates very closely the growth of actual wheat plants (Figure 2). Representative results were also gained under sub-optimal conditions and while the "plants" were respiring in the dark. Even so, we do not propose this submodel as a detailed model of a wheat plant. For our immediate purposes, however, it appears to suffice. Future BLSS model development will pay careful attention to improvements in the plant submodels employed.

The nutrient mixer. This processor is also located in the PGM. Its simple function is to refill the Nutrient Solution Reservoir with water and with nitrate (in the form of nitric acid). Thus it interfaces with the HNO_3 Reservoir and with the H_2O Reservoir. First it refills the Nutrient Solution Reservoir with the proper amount of water, and then it adds enough nitric acid to maintain the reservoir at the proper nitrate setpoint.

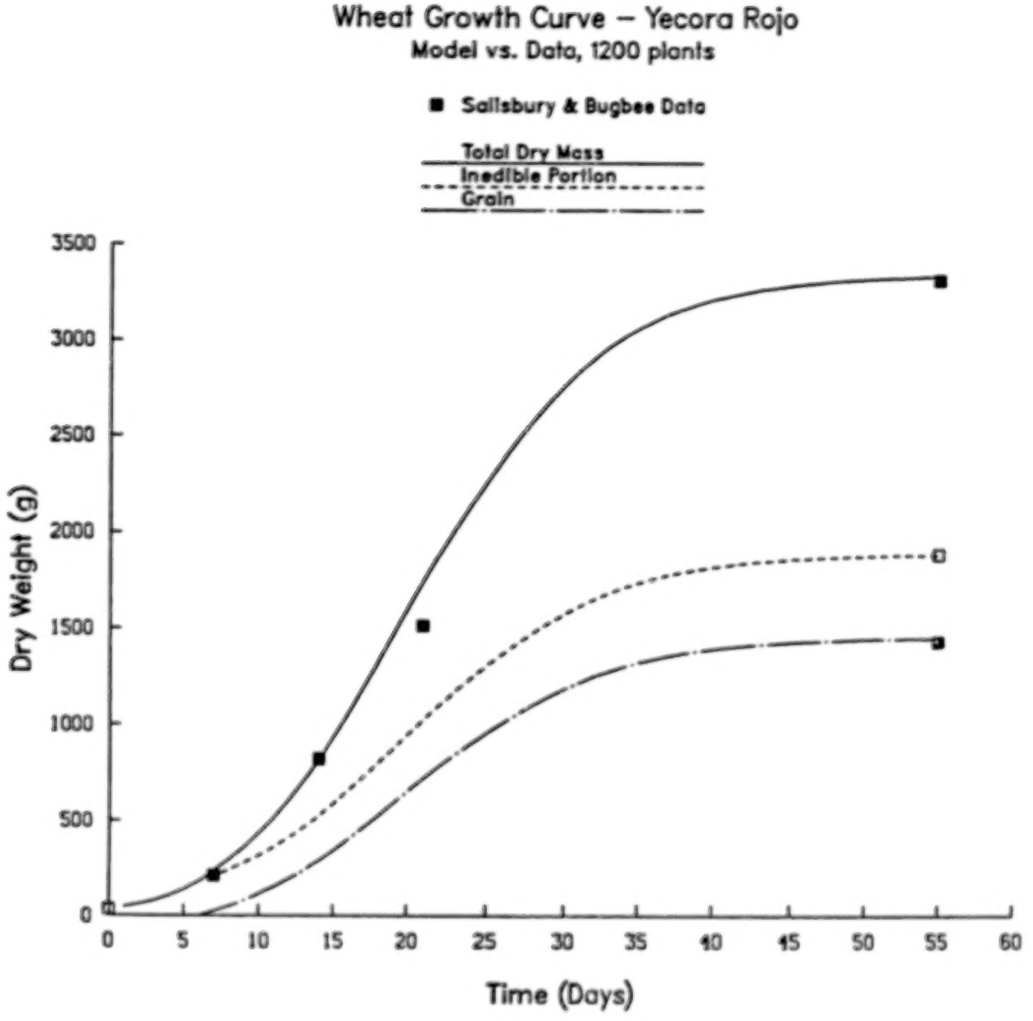

Fig. 2. Wheat growth in 1 m^2 from both actual data and the plant-growth submodel used in the BLSS Simulation Model. Growing conditions in the model were constant light, 1000 $\mu l/l$ CO_2, and 4.0 mM NO_3^-.

The crew. The Crew lives in the crew module, eat harvested food from the Plants (found in Food Storage), and drink water from the H_2O Reservoir. After the food is metabolized, the Crew adds human waste and waste water to the Waste Storage. Crewmembers typically exchange CO_2, H_2O vapor, and O_2 with the Crew Atmosphere. The crewmembers can have individual sleeping and eating schedules. Each person eats three meals a day and uses drinking water only while awake. Wash water is used over the entire 24 hour day. All crewmembers are assumed to have identical metabolisms, with no metabolic provision being made for a net change in the mass of the Crew. The life support needs of the crewmembers were gleaned from a number of different sources /11, 12, 13/, but where necessary, a conservative estimate was taken over a more stringent one. The mass of the materials used by each crewman per day are given in Table 1.

The waste processor. This processor acts to break down waste materials into their useful inorganic components. The waste processor takes in material from the Waste Storage and oxygen from the O_2 Reservoir, and returns water to the H_2O Reservoir, carbon dioxide to the CO_2 Reservoir, and nitric acid to the HNO_3 Reservoir. Our conception of the waste processor is based on Super Critical Wet Oxidation /14/, but a number of other processes are also available /15/. We have only implemented one level of waste processing in the current version of the model, with all wastes going through the same processor. Perhaps the most blatant simplification in this first version of our BLSS simulation model is that the waste processor converts 100% of the wastes into useable substances. This includes the recapture of all of the nitrogen as nitrate. One of the first improvements we will make in the model will be to add in a realistic level of denitrification, both in the waste processor and in the plant growth module nutrient solution.

TABLE 1 BLSS Crew Mass Flows (g/person/day)

Inputs	
H_2O:	
Drinking/Food Preparation Water	4577.3
Water in Food	128.3
Wash/Flush Water	18000.0
Food:	
Edible Dry Wheat	855.0
O_2:	
To Metabolize Wheat	804.6
Outputs	
H_2O:	
Water in Urine, Feces	3025.5
Metabolic Water (Vapor)	406.0
Perspiration Water (Vapor)	1680.0
Wash/Flush Water	18000.0
Solids:	
Feces, Urine, Sweat Solids	161.4
CO_2:	
From Metabolized Wheat	1092.3

The gas separator. In order to maintain atmospheric gases at their setpoints, the gas separator adds or removes O_2, CO_2, and H_2O vapor from the PGM or Crew Atmosphere. These gases are supplied from or returned to their respective reservoirs. No specific technology was envisioned for this apparatus. Future investigations with the model will also consider a system without such a device.

The system reservoirs. In the model each reservoir is implemented as either a complex (for the mixed storages) or a simple Pascal variable. In a real system the reservoirs would consist of a storage tank (or a pressure hull in the case of the atmospheres) and the accompanying distribution piping leading to it. As such, each reservoir can be thought of as a "bus" to which the appropriate processors can be attached.

SAMPLE RESULTS

In order to demonstrate the Modular BLSS Simulation Model, a number of runs were undertaken. Each of the runs discussed below were based on the same basic system conditions. A crew of 6 was specified, living in a 280 m^3 atmosphere. For the crew the air temperature was 20°, the relative humidity 50%, the O_2 level 20%, and the CO_2 level set at 350 $\mu l/l$. Food and air consumption for the crew is listed in Table 1.

All crop plantings were based on providing slightly more than 855 g/person/day over the 55 days from planting to harvest. As a result, a total of 247,500 wheat plants were grown in each run, to provide an estimated production surplus of 42.5 g/person/day. The atmospheric conditions were the same for the plants and the crew, with the exceptions that the PGM relative humidity was maintained at 70%, and the CO_2 level was maintained at 1000 $\mu l/l$. Lighting for the plants was maintained for 24 hrs/day.

The waste processor also ran for 24 hrs/day (to minimize preheat penalty), with a maximum capacity of 8.5 kg/hr wet waste. This value was sufficient to meet the total daily needs of the system, plus a small excess capacity.

The pure storage reservoirs were initially filled with arbitrarily high levels of materials, enough to guarantee sufficient supplies throughout the fluctuations of a normal year in the BLSS. For the runs below, the H_2O Reservoir initially held 10,000 kg, the CO_2 reservoir held 6,000 kg, and the O_2 and HNO_3 reservoirs were each filled with 1,000 kg. By tracing the maximum and minimum levels reached by a reservoir in any run, the necessary reservoir capacities could be determined by reference to these initial starting levels. At the beginning of all runs the waste reservoir was empty, and the atmospheres and the Nutrient Reservoir were at there respective setpoints. An initial food supply of 340 kg dry wheat was also provided, both as a food reserve and for use as seeds (7.4 kg).

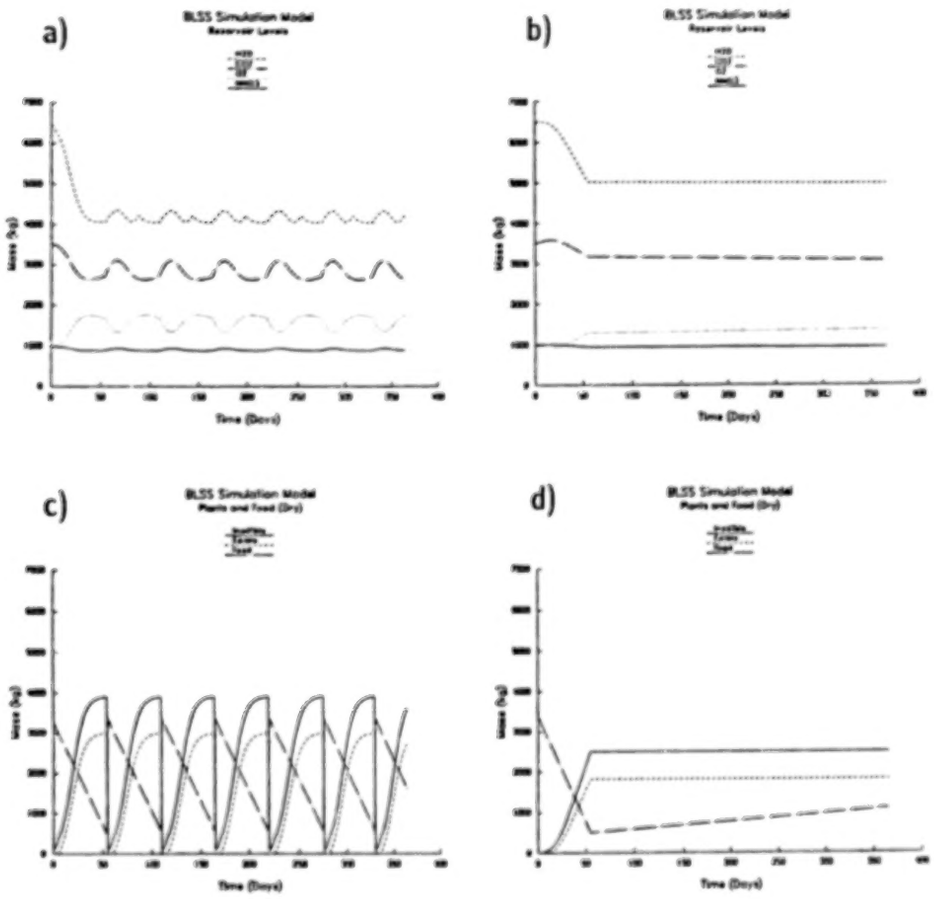

Fig. 3. Results from two planting schemes. Planting one big batch every 55 days results in the graphs shown in (a) for the reservoir levels, and (c) for the dry plant biomass in the system. These are contrasted to the results gained from planting and harvesting one batch per day (b and d). The food storage level in (d) eventually reaches the initial amount during the fifth year of system operation.

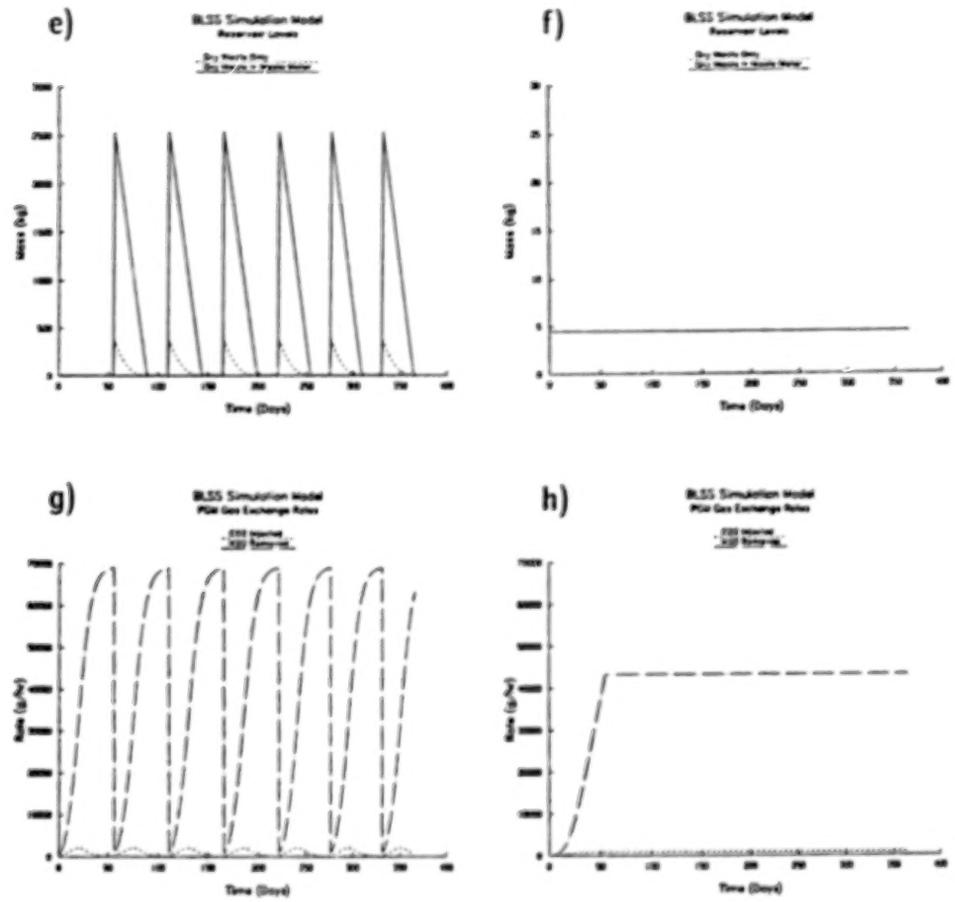

Fig. 3 (cont.) Results from one-big-batch planting are shown in (e) for the waste reservoir and in (g) for the hourly gas exchange rates. Contrasting results from the one-batch-per-day scheme are shown in (f) and (h). Note the scale difference between (e) and (f).

Planting Considerations

Two planting methods were compared. The first involved planting all 247,500 plants in one big batch, beginning on the first day and repeating the process after every harvest. The second method involved making daily plantings of 4500 plants, and after 55 days making one harvest and one planting each day. This small batch method therefore used the same number of plants as the big batch method.

The effects of planting one big batch versus 55 small batches can be seen in Figure 3. It is clear that having a daily harvest/planting cycle results in a much more stable situation in the system reservoirs, as well as in the CO_2 injection and water condensation apparatus. In fact, in the variant of the model with the PGM and Crew Atmosphere connected (Figure 1b), the small batch system only requires the *addition* of CO_2 into the single atmosphere. Even with that connection the big batch method would require both addition and removal of CO_2. Hence the mass penalty for CO_2 removal equipment would not have to be paid in such a system if the correct planting scheme is adopted.

Waste Processor Failure

Babcock et al. /4/ used a 10-day waste processor failure to investigate the dynamic interaction of system mass and storage sizes in a simple CELSS model. Figure 4 compares the effects of such a failure in a BLSS with one big batch planting to the effects in a BLSS with daily plantings. The severity of the effects of this failure on the big batch planting varies depending on the timing of the failure. It is obviously worse if the failure occurs during harvest time. This consideration does not affect the system with daily plantings/harvests. Because the storage reservoirs in these runs have a good deal of excess capacity the plants and the crew are unaffected by the waste processor failures shown here. During the failure the system lowered the level of the H_2O Reservoir by almost 2,000 kg more than is normal with the big batch planting, and by more than 1,500 kg when the daily planting scheme was used.

Crop Failure

We have also investigated the system under both planting schemes when all or a portion of the crop fails. As with the waste processor failure, in case of a crop failure the scheme with one big batch planting is much more sensitive to the timing of the failure than is the scheme with daily plantings. If the single large crop is destroyed right before harvest it is much more devastating than if a young crop is destroyed. Having the small daily plantings puts a smaller proportion of the overall crop at risk at any one time. After the BLSS has reached equilibrium with the daily planting scheme, destroying the whole crop is equivalent to a restart of the system. No shortages will take place. With the single big batch planting scheme, crop failures at certain times (e.g., at 45 days after crop planting) will cause the system to run out of food.

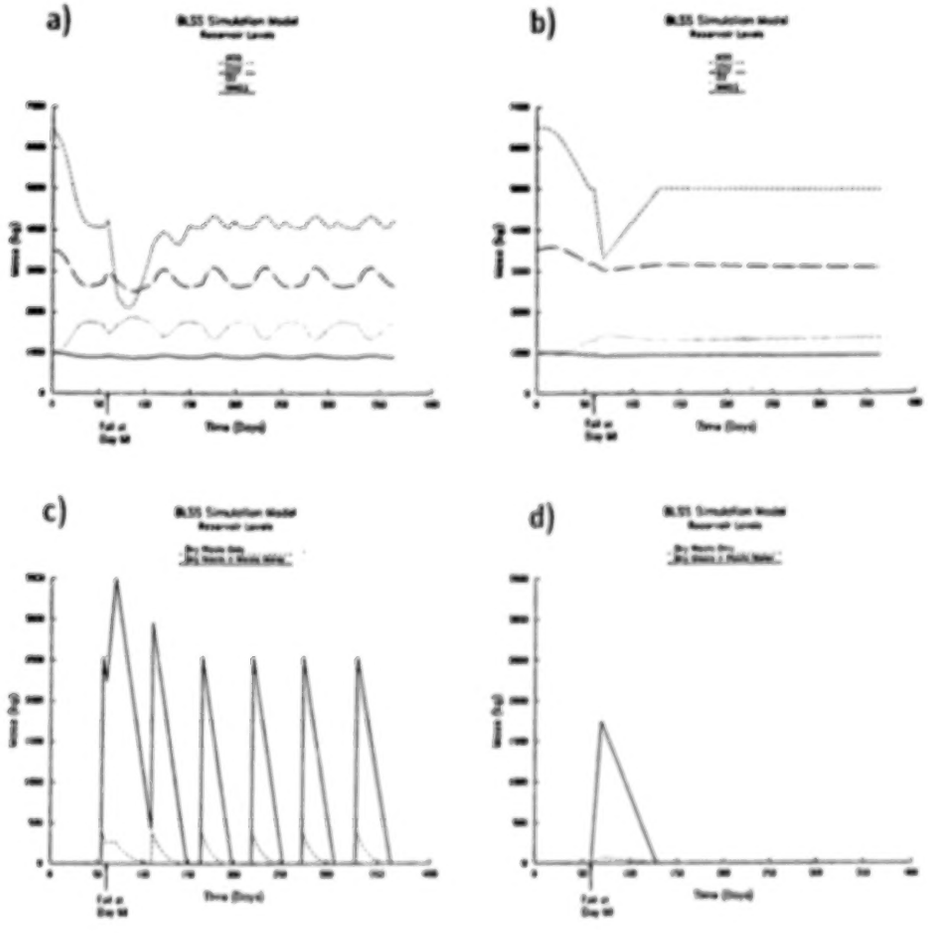

Fig. 4. Waste processor failure. The waste processor was failed at day 60 and restarted at day 70. Reservoir levels from the one-big-batch planting scheme are shown in (a) and (c). Results from the one-batch-per-day scheme are shown in (b) and (d).

DISCUSSION

One fact that is apparent in the results of the Modular BLSS Simulation Model is that such a system can be designed to be stable with only a minimum amount of dynamic control on the system processors. Control of the system is strongly dependent on control of the plants and the waste processor. If those processors remain stable and are interfaced with sufficient material buffers, then the system itself can remain stable over long periods.

Because processor stability is maintained, it is also apparent that a planting scheme that maintains a nearly constant amount of growing biomass will be more successful than the alternate scheme of large batch cultivation. This difference is maintained even under various failure modes that affect the BLSS. While common sense might also point to this conclusion, the model presented here allows us to quantify the advantages of the one scheme over the other.

The BLSS Simulation Model described here can be of use in investigating additional system. To improve the usefulness of the model in assessing different aspects of BLSS design, the model will be modified and improved to meet those needs. As was mentioned above, the model will soon be expanded to include nitrogen cycling and the effects of partial waste-processor returns on the system mass flows. In addition, the plant and crew submodels will be improved to improve our investigations into the reservoir-size boundary conditions. Eventually the scope of the model will be expanded to track other elements, including P, S, and an array of micronutrients such as K, Ca, and Mg.

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N88-12261

PRELIMINARY EXPERIMENTAL RESULTS OF GAS RECYCLING SUBSYSTEMS EXCEPT CARBON DIOXIDE CONCENTRATION

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ABSTRACT

Oxygen concentration and separation is an essential factor for air recycling in a CELSS. Furthermore, if the value of the plant assimilatory quotient is not coincident with that of the animal respiratory quotient, the recovery of O_2 from the concentrated CO_2 through chemical methods will become necessary to balance the gas contents in a CELSS. Therefore, oxygen concentration and separation equipment using Salcomine and O_2 recovery equipment, such as Sabatier and Bosch reactors, were experimentally developed and tested.

1. BASIC CONSIDERATION ON GAS RECYCLING

Fundamental functions of gas recycling in CELSS, as shown in Fig. 1. 1, are to separate each component, such as O_2, CO_2, and N_2 gases, and to concentrate and store each gas in order to supply appropriate gas concentration for human beings, plants and algae.

2. OXYGEN RECOVERY FROM CONCENTRATED CARBON DIOXIDE

Many research and development efforts have been conducted for the recovery of oxygen from concentrated CO_2 by the catalytic hydrogenization process. The Bosch reaction utilizes an iron catalyst and produces carbon (C) and water (H_2O) with CO as an intermediate product /1/. Product water is further electrolyzed to recover O_2 for animal respiration and H_2 for subsequent hydrogenization. The Sabatier reaction utilizes a ruthenium (Ru) catalyst and produces methane and water /2/. An additional CH_4 cracking process providing C and H_2 is necessary to make the Sabatier reaction comparable to the Bosch reaction /3/.

Mitsubishi Heavy Industries, Ltd. (MHI) has been involved in CELSS research for several years /4/ under the direction of the National Aerospace Laboratory and conducted the experimental program to evaluate the basic characteristics and performance of the two oxygen recovery processes.

2.1 Once Through Tests

In order to determine the basic characteristics of each reaction, once through tests of CO_2 reaction with H_2 on the catalyst are performed. Fig. 2.1 shows the schematic of the test set up.

Bosch Reaction Fig. 2.2 shows the reaction rate of CO_2 vs. reaction temperature in the first Bosch reaction, as well as the reaction rate of CO vs. temperature in the second Bosch reaction. The different nature of the two reaction rate curves suggests a phased process concept using two reactors operating at different temperature ranges to obtain higher performance in the Bosch CO_2 cracking process.

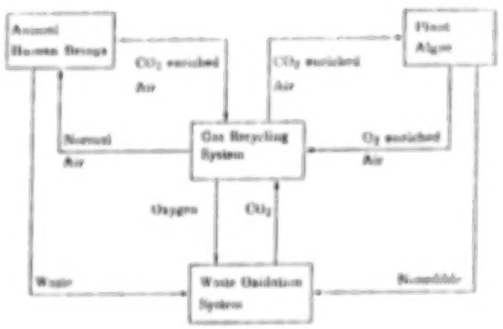

Fig. 1.1 Fundamental function of Gas Recycling System

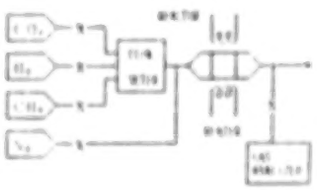

Fig. 2.1 Bosch/Sabatier Once Through Test Setup

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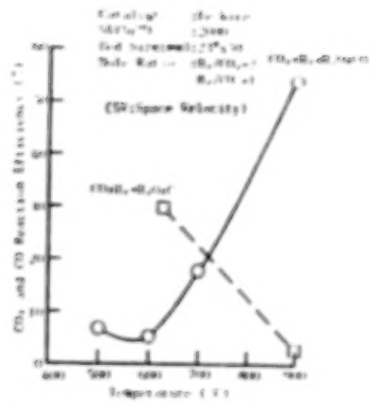

Fig. 2.2 Reaction Efficiency vs Temperature of Bosch Reaction

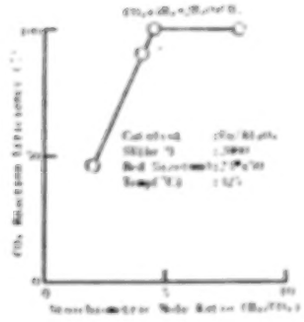

Fig. 2.3 CO_2 Reaction Efficiency vs H_2/CO_2 Mole Ratio for Sabatier First Reaction

Sabatier Reaction Fig. 2.3 shows the reaction rate of CO_2 vs mole ratio of H_2/CO_2 in the feed gas mixture of the first Sabatier reaction. Reaction rate of more than 99 % is achieved with a little bit higher ratio (4.5) than the stoichiometric value.
As for the second Sabatier reaction, reaction rate of CH_4 vs. time after start of the reaction is presented in Fig. 2.4. Very rapid degradation is observed for the catalytic reactions using Pt or Ni, while a steady and much higher conversion efficiency is observed in pyrolytic reaction on silica wool filler.

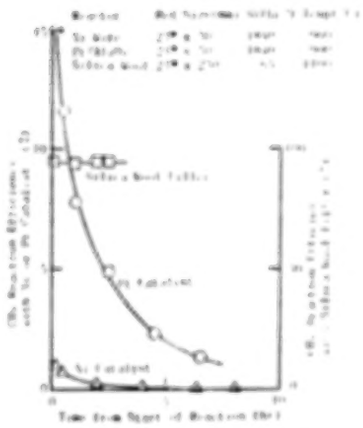

Fig. 2.4 CH_4 Cracking Efficiency vs Time for Sabatier Second Reaction

2.2 Recycle Test

A Recycle Test Apparatus was prepared based on the once through test results as shown in Fig. 2.5.

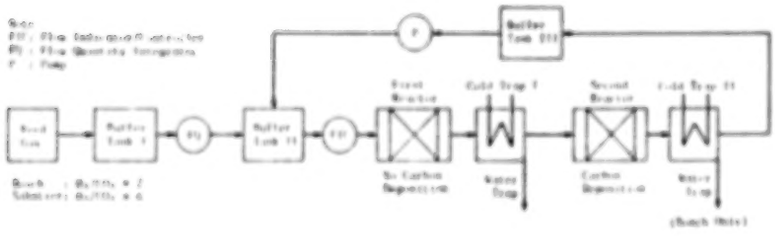

Fig. 2.5 Bosch/Sabatier Recycle Test Setup

The supply rate of mixture gas is shown in Fig. 2.6. The amounts of processed CO_2 are 0.15 kg/day and 0.42 kg/day for Bosch and Sabatier, respectively. Energy required for the above processes were estimated and the Sabatier process showed several times less energy use than Bosch process. The character of deposit carbon in the Bosch process was a loose powder form, while a hard solid block was obtained from the Sabatier reactor. This fact has an important meaning for maintainance operations to periodically extract carbon on orbit.

Thus, the O_2 recovery system with Sabatier methane cracking shows a good possibility for application in CELSS and Space Station.

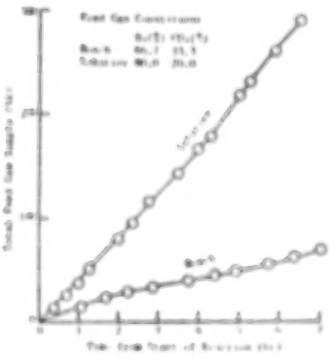

Fig. 2.6 Feed gas Supply Rate in Bosch/Sabatier Recycle Process

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3. OXYGEN SEPARATION SYSTEM USING SALCOMINE

3.1 Functions of Oxygen Separation System

Kawasaki Heavy Industries Ltd. (KHI) has been involved in CELSS research under contract with the National Aerospace Laboratory (NAL) /5/.
Fig. 3.1 shows the function of O_2 separation and concentration system, concerning the CO_2 and N_2 separation and concentration. In the Fig. 3.1, the inlet gas is mixture of O_2, CO_2 and N_2. At the CO_2 concentrater, CO_2 is separated and concentrated, and stored in the CO_2 gas bottle. At the O_2 concentrater, O_2 is separated and concentrated, and stored in the O_2 gas bottle. The residual is N_2 gas and this is stored in the N_2 gas bottle. Thus, inlet gas is separated into three (3) components, CO_2, O_2, and N_2 having high purities.

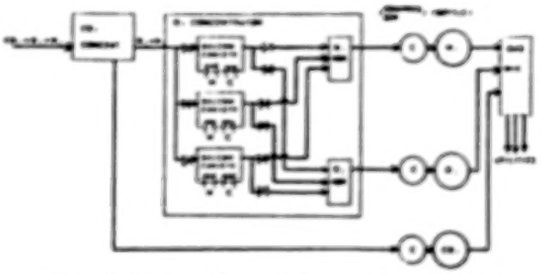


Fig. 3.1 Function of Oxygen Separation and Concentration System

There are many methods for CO_2 separation and concentration. The studies reported here are focused on the O_2 separation and concentration system using Salcomine.
Salcomine absorbs the O_2 under normal temperature (lower than about 40℃) and desorbs the O_2 under high temperature (higher than about 80℃). So, when the inlet gas is introduced into the Salcomine canister, the included O_2 is absorbed by Salcomine. After the Salcomine absorbs the O_2, the Salcomine canister is heated and Salcomine desorbs O_2 of high purity.
In the Fig. 3.1, there are three (3) canisters and each canister is operated in absorbing, desorbing and pre-cooling modes respectively. Thus, continuous O_2 separating operation can be carried out by the cyclic exchange of these operation modes.

3.2 SALCOMINE

Fig. 3.2 shows the structural formula of the Salcomine and its O_2 absorption and desorption. There are some studies on the application of Salcomine for O_2 concentration. Matsuda /6/ carried out the experimental study on the O_2 absorbing and desorbing characteristics and their variations under repeated reaction of the Salcomine, as a part of the development of Diffusive Atmosphere Control System (DACS, a kind of artificial gill), which extract O_2 from sea water dissolving oxygen.
The Present study is based on these results and is designed to get data to determine the optimum O_2 separating and concentrating system for CELSS. In this study, O_2 absorbing and desorbing performance tests of Salcomine within the canister are carried out to get the data to design a compact, light weight and lower energy consuming system.

3.3 Oxygen Absorbing and Desorbing Test of SALCOMINE

3.3.1 Test of SALCOMINE Proper. In Fig. 3.3(a), oxygen containing nitrogen gas is introduced into the equipment and Salcomine absorbs the O_2, and increases its weight. And then the Salcomine is heated and the Salcomine decrease its weight desorbing O_2. From the weight change of the Salcomine, the O_2 absorbed and desorbed is measured.
Test results are shown in Fig. 3.3(b). O_2 absorbing and desorbing capacity of Salcomine is expressed with the percentage of oxygen weight to the Salcomine weight (wt%). From Fig. 3.3(b), O_2 absorbing capacity is about 4.0 wt% for 20% of O_2 concentration.

3.3.2 Test of SALCOMINE within Canister. In Fig. 3.4, O_2 containing air is drawn by the air pump and introduced into the Salcomine canister. In the canister, O_2 in the air is absorbed by Salcomine.

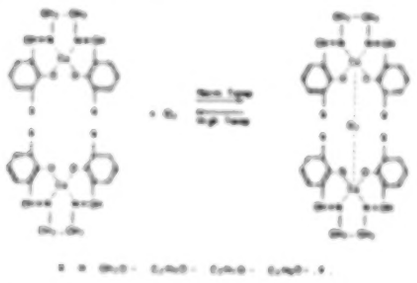
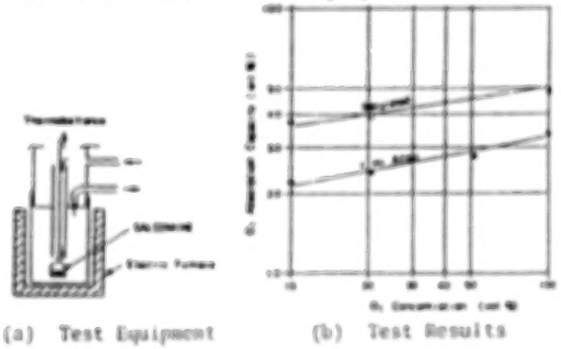


Fig. 3.2 Oxygen Absorb and Desorb Reaction by SALCOMINE



(a) Test Equipment (b) Test Results

Fig. 3.3 Characteristic Test of SALCOMINE Proper

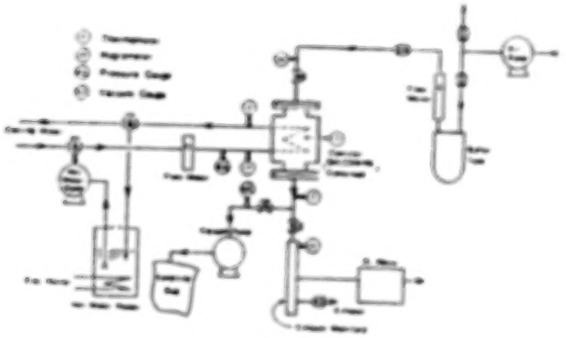


Fig. 3.4 Diagram of Canister Contained SALCOMINE Performance Test

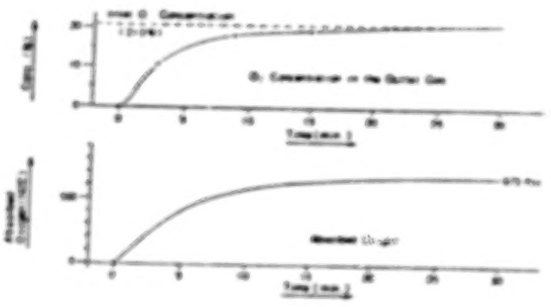


Fig. 3.5 Results of Canister Contained SALCOMINE Performance Test

And at the exhaust gas manifold, O_2 concentration is measured. During this time, The Salcomine canister is cooled. For the desorbing test, the canister is heated by hot water (90℃) and drawn by the vacuum pump. O_2 desorbed from the Salcomine is collected into the sampling bag and analysed.
Fig. 3.5 shows the results of the test. Initially, O_2 concentration in the outlet air is almost 0% because all O_2 in the air is absorbed. After a few minuits, the break-through occurs and O_2 concentration rises, and reaches to 21% which is equal to that of the inlet air. From Fig. 3.6, absorbed O_2 is 675CC and this corresponds to 2.7 wt% of O_2 absorbing capacity. On the start of O_2 desorbing test, the vacuum pump is operated to purge the air inside the test equipment. The canister is then heated. And is drawn by the vacuum pump. The drawn gas is measured 710 CC, and O_2 concentration in this gas is measured 91.4%. So, desorbed O_2 becomes 649 CC.

4. CONCLUSION

As a part of CELSS Gas Recycling System Development, preliminary experimental studies were conducted for the processes of O_2 recorvery from concentrated CO_2 and of O_2 separation and concentration from exhaled gas of animal, plant etc.
Sabatier process for the regenerable O_2 recovery from CO_2 shows a good possibility compared with Bosch process in its higher efficiency of O_2 recovery and easier handling of product carbon. From the O_2 separation and concentration tests using Salcomine, oxygen absorbing and desorbing characteristic data are obtained as the fundamental design data.
The above results can be used in the planning of a complete and stable gas recycling system required in CELSS. We hope to continue our research and development effort for the expansion of the human frontier in Space.

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N88-12262

VAPOR COMPRESSION DISTILLER AND MEMBRANE TECHNOLOGY FOR WATER REVITALIZATION

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ABSTRACT

Water revitalization for a space station can consist of membrane filtration processes and a distillation process. Water recycling equipment using membrane filtration processes was manufactured for ground testing. It was assembled using commercially available components. Two systems for the distillation are studied; one is an absorption type thermopervaporation cell and the other is a vapor compression distiller. Absorption type thermopervaporation able to easily produce condensed water under zero gravity was investigated experimentally and through simulated calculation. The vapor compression distiller was studied experimentally and it offers significant energy savings for evaporation of water.

INTRODUCTION

Engineering and experimental studies on water recycling systems for space stations have been conducted by the NAL (National Aerospace Laboratory), Hitachi, Ltd., and the Sasakura Engineering Co., Ltd. The system is applicable to purification of waste water from the crew and from life science experiments in the space station /1/. The equipment for ground-based experiments of a three-step filtration process was manufactured by Hitachi, Ltd. /2/. Two types of distillation under study are presented: the absorption type thermopervaporation membrane evaporation by Hitachi, Ltd. /3/ and vapor compression distillation (VCD) by the Sasakura Engineering Co, Ltd.

The water purification process consists of a three-step filtration involving prefiltration, ultrafiltration and reverse osmosis; activated charcoal removes solutes, such as trace organic materials left in the previous filtration process; and distillation which further purifies water treated through the filtration processes in order to produce potable water. In addition for storing the treated water, an ultraviolet sterilizer is provided to prevent the growth of bacteria.

EQUIPMENT FOR GROUND EXPERIMENTS

To make an optimal system configuration of water revitalization for space stations or in a CELSS, various experiments, especially regarding water quality in each process, should be conducted in order to figure out technical difficulties and to check and confirm maintenability, safety and reliability. For this purpose, equipment for ground experiments for water recycling was manufactured by Hitachi, Ltd. for the NAL. This experimental equipment consists of three-step filtration and the activated charcoal.

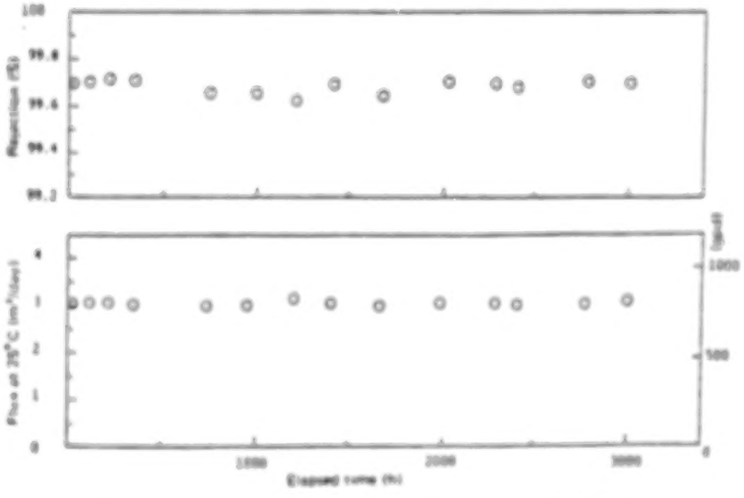


Fig. 1. Brackish water desalination performance of NTR-7199 membrane.

The processing performance meets requirements for the EDC Japanese life science missions of an average of 2.1 ℓ of water a day including 0.5 ℓ potable water. However, the actual performance exceeds these requirements; the processing rate is 10 ℓ/h for ultrafiltration and 0.6 ℓ/h for reverse osmosis, because the components used have higher specifications. The ultra-

filtration module and the reverse osmosis module were supplied by the Nitto Electric Industrial Co., Ltd. (Ultrafiltra-tion Module: NTU-3250-C1Rc [spiral wound type] and Reverse Osmosis Module: NTR-7199-92Bx [capillary type]). The modules were remodeled from commercial products into products with a smaller processing capability in order to adjust them to the pumps used. Each filtration process has a different processing rate, except the activated charcoal which depends on the permeate rate from the reverse osmosis module, so the system is operated in a batch processing manner.

Experimental studies using this experimental equipment will be made in the near future. Data of a membrane of the same type as the membranes used are shown in Fig. 1, where water flux and salt rejection remained at constant high values throughout the 3000-h test /4/.

ABSORPTION TYPE THERMOPERVAPORATION

The thermopervaporation process uses a hydrophobic membrane of porous polytetrafluoroethylene (PTFE) which was chosen because it permeates vapor, not liquids. This membrane is under development in cooperation with Nitto Electric Industrial Co., Ltd. The permeate flux is proportional to the vapor pressure difference (ΔP) between the feed and the permeated water, expressed by $F = K \cdot \Delta P$, where K is the proportionality constant (permeate coefficient) representing the permeate rate. The electrical conductivity of the treated water is below 10 μS/cm, compared to 48 mS/cm of the feed, and the rejection is more than 99.99 %, irrespective of the vapor pressure difference ΔP.

In the former type of thermopervaporation /1/, the problem arose from how to collect the condensed water from the cooled plate in micro gravity. It is easy to collect such water on earth due to the existence of gravity. To resolve this problem the absorption type thermopervaporation is proposed for easy water-gathering, because the permeated vapor is absorbed into the product water. In this system, the feed on the high temperature side of the membrane is vaporized, permeated through the membrane pores, and then absorbed into the cooled permeate which is circulating.

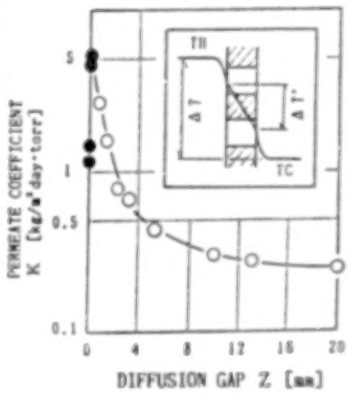


Fig. 2. Experimental results of thermopervaporation

Experimental Study

Experimental data from absorption type is compared to the earlier type process and is shown in Fig. 2, in which black circles show the results of absorption type thermopervaporation corresponding to a diffusion gap of zero.

The value of K is scattered in the range of 1 to 5 kg/m²day·torr. This can be explained by the fact that the bulk temperature difference (ΔT) differs from the temperature difference ($\Delta T'$) contributing to the real vapor transfer through the membrane due to the temperature drop near the membrane, as shown in Fig. 2. Moreover, the rate of vapor transfer in the membrane depends on its thickness and porosity. Thus, for the thicker the membranes or the smaller the voids, the more resistant the membrane becomes to the permeate flux. Thus K is affected by the material and structure of the membrane and the operating conditions. From these experimental results, at least 1 kg/m²day·torr was verified. However, in this method, the loss in the sensible heat transfer increases in comparison with the former method. Therefore, further evaluation is needed to reduce this thermal loss.

Theoretical Study

The theoretical study was made based on a model with a heat exchanger (see Fig. 6), which is provided for enegy recovery in order to reduce power consumption. Calculations were made concerning the necesary heat input (Q/Q_0), the circulation rate (W) of the feed, the effective heat transfer area (Ahe), and the effective membrane area (Am) versus the temperature difference (ΔT) between the feed and cooled water, the temperature difference (ΔTi) between the inlet and outlet of the feed, and the membrane thickness (δ), where Q_0 is the heat input in case of no heat loss, expressed as $Q_0 = F' \lambda Am = 0.25 \lambda$ ($F' Am = 0.25$ kg/h, F': permeate rate, λ: latent heat). ΔTi is assumed to be the same as for the cooled water, since the circulation rate of the feed is the same as that of the cooled water and the temperarure difference in the heat transfer in the heat exchanger is fixed at 5 ℃. In this calculation, the following parameters are predetermined, thermal conductivity of the membrane = 0.0634 kcal/mh℃, permeate = 0.25 kg/h, feed water = 0.28 kg/h, concentrate = 0.03 kg/h.

Figs. 3 to 5 show the calculated results. As shown in Fig. 3, ΔT is best when set to 5 ℃, specifically from the viewpoint of minimum thermal loss. A temperature THin = 90 ℃ of the feed at inlet is desirable for efficiency.

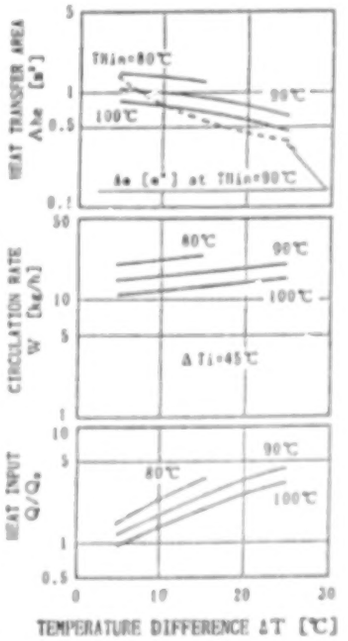
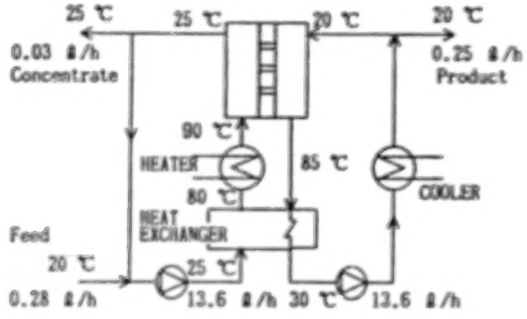


Fig. 3. Influence of ΔT on Q, W and Ahe.

Other data in Figs. 4 and 5 are obtained using these values. In Fig. 5, ΔTi is chosen as 65 ℃, being seen as suitable from the results in Fig. 4. As the results show, an example of the optimum operating conditions of the absorption type thermopervaporation is demonstrated by Fig. 6, where a membrane thickness of 0.08 mm is selected because this thickness is easily available. If 0.2 mm thickness is selected, the heat input can be reduced by 40 % as seen from the data in Fig. 5.



Am: 0.19 m², δ: 0.08 mm, Q: 154 kcal/h, Ahe: 0.15 m²

Fig. 6. Suitable conditions of thermopervaporation process

From these simulated calculations, the most important problem is seen to be heat loss due to the sensible heat transfer. When this approach is used in different water recycling systems, the optimum operating conditions have to be decided on the basis of reducing heat loss as the primary concern.

VAPOR COMPRESSION DISTILLATION (VCD)

VCD is another candidate as a distillation process for the water revitalization in the space station, since it offers a low specific energy consumption.

Principles and Features of VCD

Fig. 7 shows a typical example of VCD used for seawater desalination. The feed water is warmed up prior to evaporation. The vapor generated in the evaporator is compressed by a blower, which plays role of a heat pump to upgrade the vapor, and is then fed into the tubes, becoming the heat source for the evaporation. Thus, low specific energy consumption is obtained by the energy recovery in the preheating and the evaporation steps.

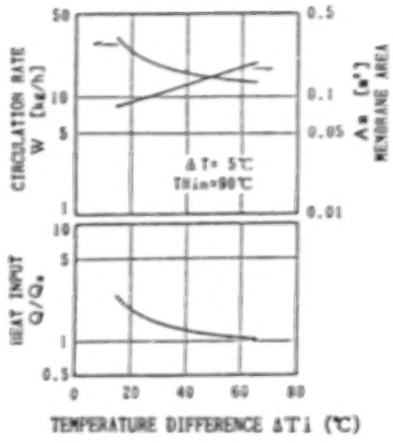


Fig. 4. Influence of ΔTi on Q, W and Am

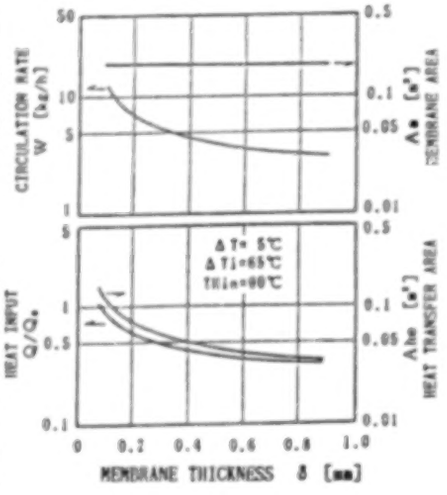


Fig. 5. Influence of δ on Q, W, Ahe and Am.

In applying VCD to space stations, artificial gravity is needed for gathering liquids. Fig. 8 shows a concept of a VCD unit to be operated in micro gravity. The inside of the casing is kept at a vacuum by a vacuum pump. The two drums, fixed to each other, and the blower are rotated by the motor. The feed water forms a film on the surface of the inner drum due to centrifugal force and evaporates. The generated vapor in the inner drum is compressed, introduced into the gap between the two drums and then condenses on the inner drum surface, discharging energy for evaporation of the feed. The residual brine and the condensed distillate flows along the tapered drum surfaces and is collected at the troughs.

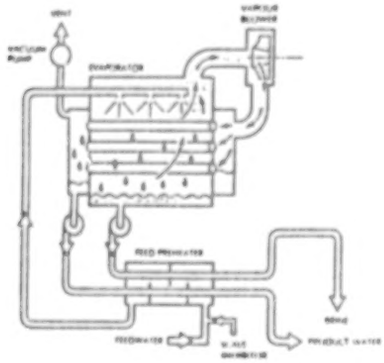


Fig. 7. VCD for seawater desalination

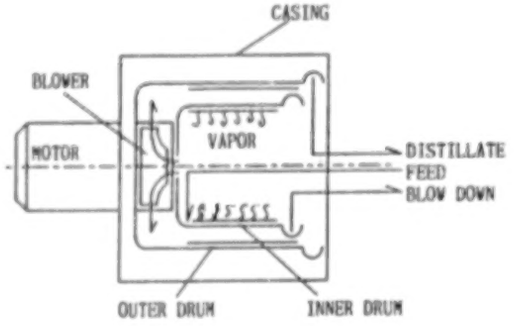


Fig. 8. VCD concept for space use

Experimental Study

An experimental model having a 450 mm diameter and a 620 mm length was made to produce one liter of water per hour, a chart of which is shown in Fig. 9. The feed is preheated in the water bath and the measured amount is sucked by the distiller which is kept in vacuum conditions. The product water and the residual brine are extracted by the twin element pump. The temperature, the pressure and the flow rate are monitored at necessary points. A level alarm is installed in the inner drum to prevent carry over of the feed water to the blower. The distiller employs a single motor for both drum rotation and blower drive. Accordingly, the rotation speed of the motor relates to both the behavior of the brine film on the inside surface of the inner drum and the blower performance.

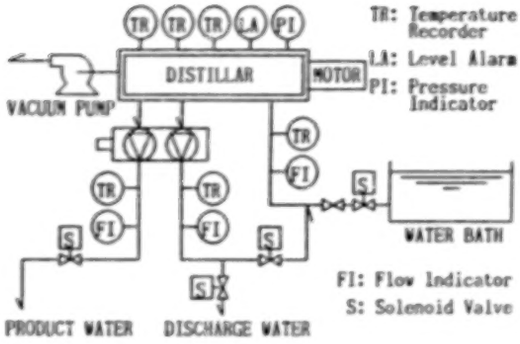


Fig. 9. Flow chart of the experimental VCD model

Fig. 10 shows experimental data of the distillate production rate versus the temperature difference between the blower inlet and outlet (△—△). The temperature in the inner drum was kept at 29 ± 0.3 ℃ during this test. The distillate production rate increses as the rotation speed increases, but the rate of increase is a little less than the increase rate of the temperature difference between the inlet and outlet of the blower. It is presumed that the temperature at the outlet of the blower is superheated due to compression and the temperature difference described in Fig. 9 is not the difference of saturation temperature. Another reason would be that the pressure drop through the vapor path becomes large as the distillate production (vapor flow rate) increases, and the actual saturation temperature difference across the heat transfer surface becomes smaller than the superficial temperature difference as the vapor flow rate increases.

Experimental results shown in Fig. 10, (○---○) in the case of desuperheating, are obtained when desuperheating water is introduced into the suction of the blower. It shows that the relationship between the rate of increase in the distillate production becomes large due to the desuperheating effect.

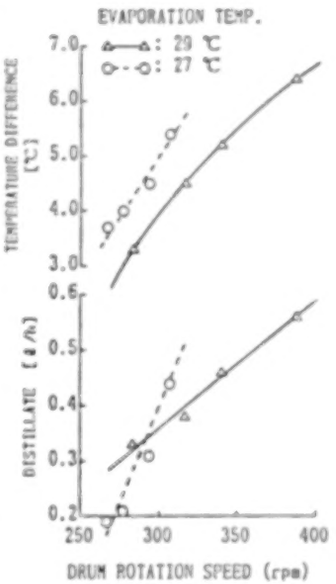


Fig. 10. Effect of drum rotation

The evaporation temperature can be controlled by adjusting the feed water temperature. Fig. 11 shows the distillate production and the temperature difference between the blower inlet and outlet versus the evaporation temperature. The distillate increases as the evaporation temperature increases. The reason for this phenomenon is that the heat transfer coefficient increases with increases in the evaporation temperature. Since the temperature difference decreases with elevation of the evaporation temperature, the rate of increase of the heat transfer coefficient is presumed to be of significance. Another reason may be due to a decrease of the pressure drop across the vapor path, in other words, increase in the effective temperature difference across the heat transfer surface due to the increase in the density of the vapor as the temperature increases.

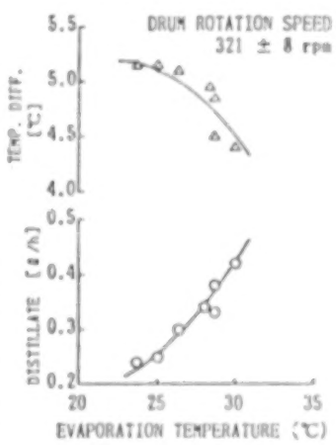


Fig. 11. Dependence of distillate and temperature difference on evaporation temperature.

CONCLUSIONS

Water revitalization for the space station can be realized by a combination of the filtration processes and a distillation process. Detailed systematic studies will be performed using ground-based equipment. There are two candidate distillation process; thermopervaporation and VCD. The absorption type thermopervaporation was studied through simulated calculations, so that an optimum condition were obtained. The VCD with centrifugation was experimentally confirmed to be a candidate for space use.

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N88-12263

FUNDAMENTAL STUDY ON GAS MONITORING IN CELSS

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ABSTRACT

A mass spectrometer and computer system was developed for conducting a fundamental study on gas monitoring in CELSS. Respiration and metabolism of the hamster and photosynthesis of the Spirulina were measured in a combination system consisting of a hamster chamber and a Spirulina cultivator. They are connected through a membrane gas exchanger. Some technical problems were examined.

In the mass spectrometric gas monitoring, a simultaneous multi-sample measurement was developed by employing a rotating exchange valve. Long term precise measurement was obtained by employing an automatic calibration system.

The membrane gas sampling probe proved to be useful for long term measurement. The cultivation rate of the Spirulina was effectively changed by controlling CO_2 and light supply. The experimental results are helpful for improving the hamster-spirulina system.

INTRODUCTION

A fundamental study on gas monitoring in CELSS was performed using a cmputerized mass spectrometer system. O_2 gas exchange and water consumption are of basic importance in CELSS. N_2 fixation and release are also of concern. Several kinds of organic trace gas vapours, some of which are toxic, should also be monitored./1/

In this work, a mass spectrometer computer system was developed for a basic study on CELSS. A brief description of the measuring system, some measuring results on the hamster and Spirulina system are presented. Also some technical problems will be discussed.

DISCRIPTION OF THE MEASURING SYSTEM

The measuring system is illustrated in Fig. 1. Inlet and outlet gas flows, concentrations and temperatures of the Spirulina cultivator, hamster chamber and fermentors are simultaneously monitored. O_2 uptake $\dot{V}_{O_2}$ and CO_2 production $\dot{V}_{CO_2}$ of the animal (including human), and photosynthetic O_2 production, CO_2 consumption and the related physiological factors are calculated and examined in real time.

Mass Spectrometer-Computer System: The mass spectrometer developed is a compact magnetic type. In ordinary use, O_2, CO_2, Ar, N_2 and H_2O are simultaneously measured by the fixed collectors, CH_4, NH_3 and C_2H_5OH can be measured using extended collectors. The voltage scanning system is available for other trace gas analysis. In ordinary use, changes of 0.005% concentration is measurable in long term monitoring by employing automatic calibration. Sample gas is compared to the reference in each cycle, while changes of 0.05% a day is observed in direct reading of the mass spectrometer./3/4/
Direct gas sampling through a small capillary is conventionally employed. In this case, the response of the output to the change of concentration is as high as 30 to 50 ms in 63% rise with sampling rate of 30 ml/min for dry gases. Such a rapid response is useful in the study of human respiration physiology ./2/

The membrane coated sampling probe shown in Fig.3 is also available. The sampling rate is neglegible in this case, while the response is as low as 100 s in rise time of 90%./3/ However, the response may be sufficient for long term gas monitoring.

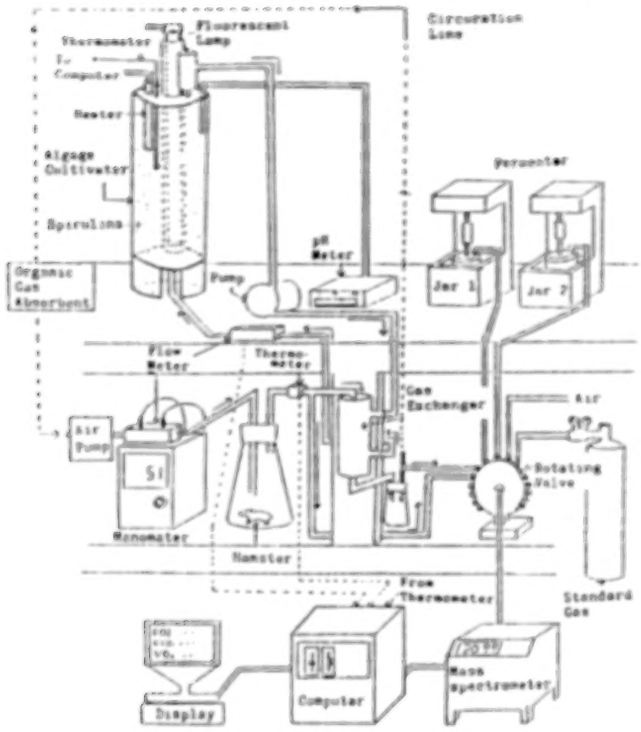

Fig.1. Experimental arrangement. Metabolism and photosynthesis were studied in the hamster and Spirulina system

Computer system: The computer system (CPU: 10/sp,Microeclipse Hard Disk Memory 15 MB) was designed to perform the following functions:
(1) controlling sample selecting (or exchanging) valve,
(2) data entry and storage,
(3) online and offline calculations,
(4) display of the measured results on CRT, printer and plotter,
(5) send the measured informations to the culture conditioner,

10 samples can be measured refering to the standard gas by employing the rotating exchange valve.

RESULTS AND DISCUSSION

Measurement of hamster metabolism

The metabolism of the hamster was measured by monitoring inlet and outlet gas concentrations, flow and temperatures. The measured results, FO_2, FCO_2, VO_2, VCO_2 and RQ are shown in Fig.2,A-C and carbohydrate(CHO) and FAT consumption and metabolic energy production are in Fig.3,A-E. The chinese hamster tested was 34.5g weight and $\dot{V}O_2$ was 2.25 ml/min (0.065 ml/g min) in average for an hour. Instantaneous $\dot{V}O_2$ varied from 1.32 to 3.14 ml/min according to the state of activity. The washout time constant of the hamster chamber is 2.7 min. The same kind of measurement is shown in Fig.4. This latter measurment was performed by employing a membrane sampling probe. The sampling rate is almost neglectible comparing to the ordinary capillary probe. This probe is also available for the direct sampling of dissoved gases.

Combination of hamster chamber and Spirulina cultivator

The exhaust gas from the hamster chamber and the water diluted gas in the Spirulina cultivator are exchanged through the membrane gas exchanger. FO_2, FCO_2 and FN_2 were continuously measured at the inlets and outlets of the hamster chamber and the gas exchanger. The result is shown in Fig.5. The extreme change in concentration at the outlet of the hamster chamber (inlet of the gas exchanger) was stabilized by the buffer effect of the water broth in the Spirulina cultivator. In this case, oxygen production rate (activity of photosynthesis) was improved to 0.4 ml/min, for a liter of the broth, by supplying expired CO_2 of the hamster, from 0.08 ml/min/1 liter broth 0.08 ml/min/1 liter was observed for the room air supply.

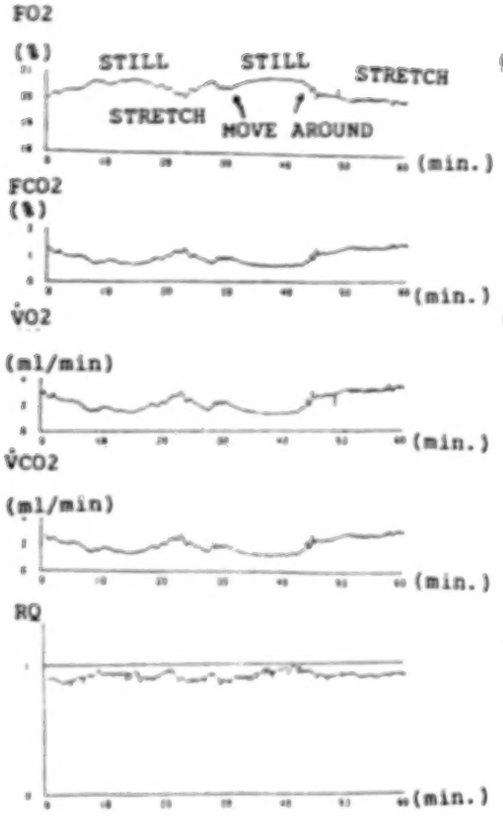

Fig.2. Continuous measurement of FO_2, FCO_2, $\dot{V}O_2$, $\dot{V}CO_2$ and RQ. The measurement was performed by employing the ordinary capillary probe.

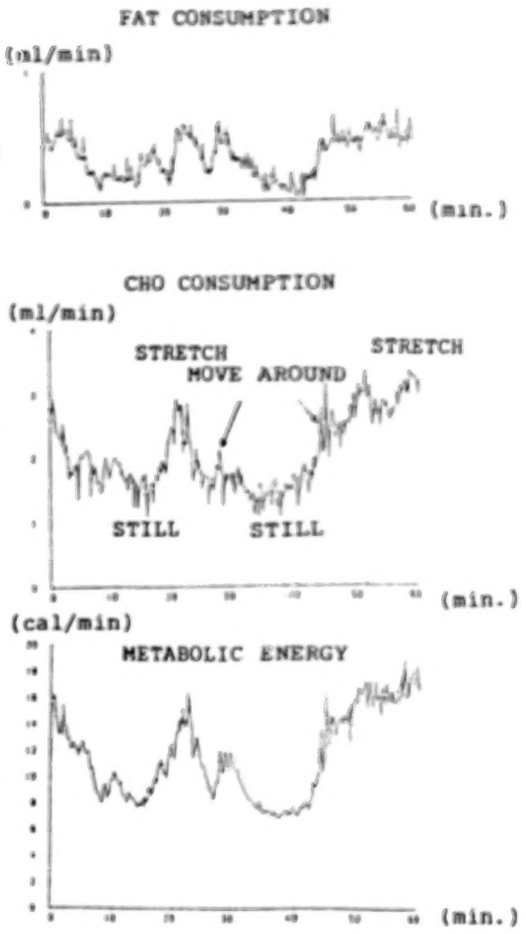

Fig.3. Simultaneous measurement of metabolic factor.

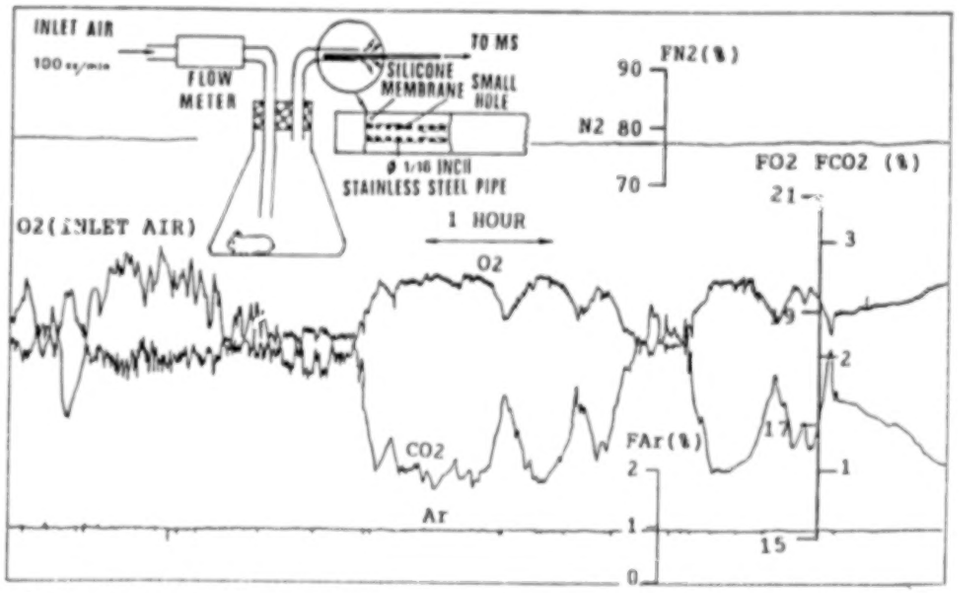

Fig.4. Over night monitoring of the exhtust gas concentration from the hamster chamber. The measurement was performed by employing a silicone membrane probe.

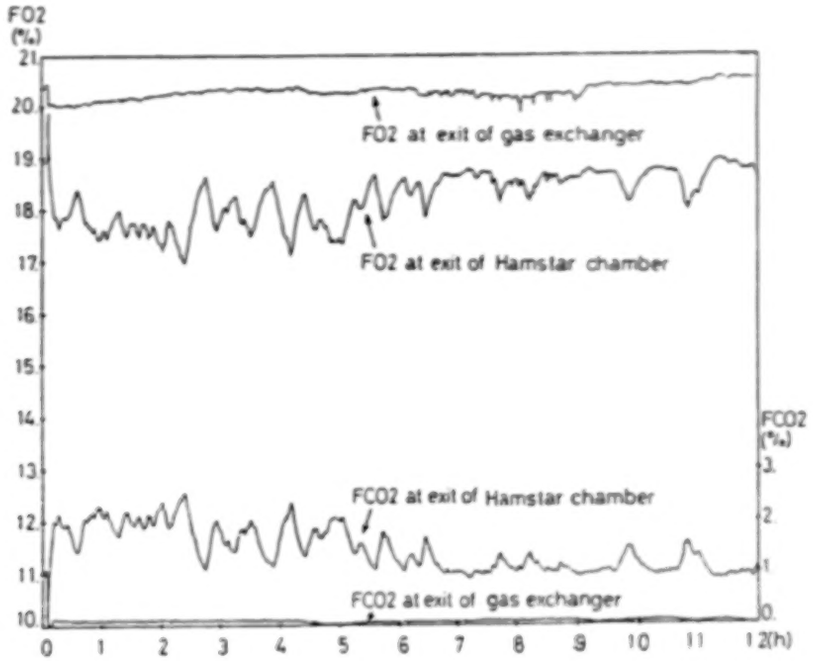

Fig.5. Continuous measurement of gas exchange in the hamster and Spirulina system. The inlet and outlet gas concentrations of hamster chamber and the gas exchanges of Spirulina system were simultaneously measured.

Considerable improvement of O_2 generation was observed by supplying expired CO_2 instead of room air. O_2 generation is, to some extent, controlled by the rate of supplyed CO_2. When lights were completely turned off, O_2 output completly ceased in 90 min. This lag time may be equivalent to the washout time of the Spirulina cultivator itself. Concerning the mass spectrometric measurement, the multi-gas sampling system and the automatic calibration proved to be useful for long term precise gas monitoring in CELSS. However, this method is possible to be employed for the ordinary cappillary sampling probe, and a considerable sampling rate is needed (at least 10ml/min) in the completely closed small size simulation system, the membrane probe may be more useful because of its negleqible small sampling rate. Further technical improvement, however, is needed to make it practical .
The considerations mentioned will help us to improve the design and control of the hamster-spirulina system.

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N88-12264

THE APPLICABILITY OF THE CATALYTIC WET-OXIDATION TO CELSS

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ABSTRACT

The wet-oxidation catalysis of Au, Pd, Pt, Rh or Ru on a ceramic honeycomb carrier was traced in detail by 16 to 20 repetitive batch tests each. As a result, Pt or Pd on a honeycomb carrier was shown to catalyze complete nitrogen gasification as N_2. Though the catalysts which realize both complete nitrogen gasification and complete oxidation could not be found, the Ru+Rh catalyst was found to be most promising. Ru honeycomb catalyzed both nitrification and nitrogen gasification.

PREFACE

Wet-oxidation is the reaction in which soluble or suspended organic materials are oxidized in a pressure vessel at a temperature of 120 to 374℃ and a residence time of several minutes to hours in the presence of oxygen and liquid water. The reaction can be carried out both in a batch system and in a continuous system.

The main advantage of wet-oxidation, when applied to the waste management system for CELSS (closed-ecological-life-support-system) in a spaceship, is in that, as a type of oxidation reactions, it can produce carbon dioxide, which is essential to plant photosynthesis, from the raw material of organic waste. In a wider sense, as it is a mineralization reaction, it can also play a part as a re-distribution center of elements to each unit of the CELSS. The other advantages of wet-oxidation are (1) no biomass reproduction, (2) the stability of the reaction, (3) the stoichiometrically required amount of oxygen to be introduced, (4) short retention time and small insulation space, (5) no additives, (6) no bacteria or virus, (7) heat recovery and so on. Among these advantages, the first point is the most important and the 4th point is also important in the initial development stage of CELSS.

But wet-oxidation has also disadvantages. In a previous report /1/, the author showed that:
(1) In wet-oxidation without catalysts, non-oxidizable acetic acid is inevitablly produced as a by-product and complete oxidation cannot be carried out. This prevents the wet-oxidation system from playing a part as the re-distribution center of CO_2 and elements in CELSS.
(2) In wet-oxidation, the organic nitrogen in raw material is transformed exclusively to ammonia and not to nitrogen gas (N_2) or nitrate form, both of which are the main chemical forms of nitrogen needed for CELSS.
In addition, the authors showed the possibility that these disadvantages may be overcome by the use of catalysts.

In the application of wet-oxidation as a waste management system for CELSS, complete oxidation and chemical form control of nitrogen are necessary. From this point of view, the authors will show in this paper the wet-oxidation catalysis of Au, Pd, Pt, Rh or Ru on a ceramic honeycomb carrier, which were proven to be useful as catalysts in the preliminary experiment /2/.

EXPERIMENTAL METHOD

Reactor and raw material: Each experiment was carried out as a batch test using an autoclave with a volume of 580 ml. The filtrate of wet-oxidized-sewage-sludge obtained from the wet-oxidation facility of Yokohama-City-Northside-Sewage-Treatment-Plant was used as the raw material. The reason why soluble raw material was used instead of suspended material is that suspended materials generally poison the active points on the surface of a catalyst, and that it appeared to make it easier to select the best catalysts.

After the raw material was introduced to the autoclave, it was heated up. The temperature

reached the designated value in 30 min. The reaction times indicated hereinafter refer to the time at the designated temperature and do not include the time needed for heating. At the end of the desired reaction time, the autoclave was drenched in water to stop the reaction.

Experiment 1: The catalytic properties of Au, Pd, Pt, Rh and Ru, each of which is supported on the ceramic honeycomb, were examined and the changes of the properties with repetition of batch tests were traced. 20 batch tests were carried out with each catalyst. In each test, fresh raw material was introduced to the reactor. By using carriers, the surface area of each compound is increased and thus the number of active sites on it increases. In addition, the seperation of the reactor output from the catalyst is also made easier. Moreover, the catalysts can be used repeatedly and the effusion of catalyst from the reactor is minimized.

A Pt catallyst with a cell number of 32.5/cm^2, a cross-section area of 5x5 cm and a height of 4 cm was used. The quantity of the Pt carried was 1.5 g per liter of the apparent volume of a honeycomb. When tested, it was laid on the bottom of the reactor. For catalysts except Pt, the cell number was 28.4/cm^2 and their shape was cylindrical with a diameter of 60 mm and a height of 50 mm. The quantity of the noble metal carried was about 2 g/l of the apparent volume of the honeycomb. It had a hole with a diameter of 8 mm at the cross-sectional center. The vertical shaft of the stirrer without a propeller was used for suspending the catalyst through the 8 mm hole mentioned above. The stirrer shaft was not moved in the experiments.

Each batch test was carried out at a temperature of 260°C, a pressure of 75 kgf/cm^2 (7.35 MP) and a time of 60 min. The stoichiometrically required amount of oxygen was added to the reactor in case of the Pt catalyst and 1.5 times of its amount was introduced in the case of the other catalysts.

Experiment 2: The combined effects of elements were examined using the Ru and Rh catalysts. Their shape were the same as those of Ru, Rh, Pt and Pd in Experiment 1. The total quantity of Ru and Rh carried was 3 g/l of the volume of catalysts. The weight ratio of Ru to Rh was 1 to 3, 1 to 1 and 3 to 1. 16 batch tests were carried out. The reactor temperature, pressure and time were also the same as those in Experiment 1. 1.5 times the amount of oxygen to oxidize raw material completely was added to the reactor.

RESULTS

Experiment 1: Figures 1 and 2 show the relation between the repetition times and COD in the reactor output when Ru, Rh, Pd or Pt on a honeycomb carrier was used. COD of the raw material was 14.4 g/l and COD of the reactor output without catalyst (control) was 5.87 g/l.

In case of Au, it was entirely released from the honeycomb carrier during the first batch test. COD and Kjeldahl nitrogen in the reactor output was shown to be the same as those of the control. The bare ceramic honeycomb without Au was put in the reactor with the same raw material as in Experiment 1 and wet-oxidized under the same experimental conditions. As a result, it was discovered that the ceramic carrier itself will not catalyze either oxidation or chemical form transformation of nitrogen.

In the case of Rh, COD of the reactor output was almost constant regardless of the number of times repeated and showed a value about half that of the control, that is, about 3 g/l. As COD of the raw sewage sludge treated in the wet-oxidation facility of Yokohama City was about 40 g/l, this value of 3 g/l accounts for 7.5% of the COD of the raw sewage slude of Yokohama City. In the case of Ru, COD is the smallest at first among the 4 catalysts, then increases gradually with repetition and approaches the control. In the case of Pd, COD is secondarily the smallest at first and then increases with repetition and COD approaches a constant value, approximately about 3/4 of the control. In the case

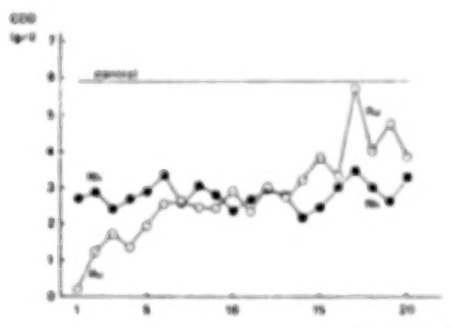

Fig. 1. The effects of the Rh or the Ru catalyst on the COD reduction

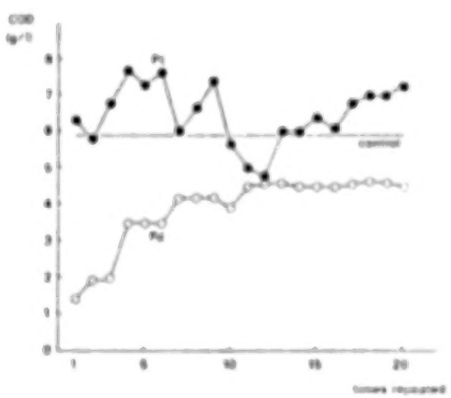

Fig. 2. The effects of the Pt or the Pd catalyst on the COD reduction

of Pt, COD repeats the increases and decreases with the repetition of the batch tests, but its change occurs near the value of the control. Therefore, it was proven that Pt honeycomb, in this experiment, does not catalize oxidation.

Figures 3 and 4 show the nitrogen quantity in the liquid of the reactor output when Rh or Ru honeycomb was used, respectively. The total nitrogen of the control was 1480 mg/l. In the case of Rh, the quantity of nitrate nitrogen is constant and small, regardless of the repetition number. On the other hand, the quantity of Kjeldahl nitrogen was very small at first, then increases and approaches a constant value after being repeated five times. The constant value occupied about a half of the control. The difference between the control and the sum of Kjeldahl and nitrate nitrogen is considered to be gasified as nitrogen gas (N_2). In the case of Ru, Kjeldahl nitrogen is very small in quantity and nitrate nitrogen is very large in quantity. Though nitrate nitrogen was so small in quantity during the first batch test, but increased suddenly after being repeated two times, and thereafter, maintained a value of about half of the control. In the case of Pd, the quantity of kjeldahl nitrogen in the liquid ranged from 1.8 to 35.3 mg/l and that of nitrate nitrogen ranged from 13.8 to 88.5 mg/l. On the other hand, in the case of Pt, Kjeldahl nitrogen ranged between 29.0 and 192 mg/l in quantity and nitrate nitrogen was not detected. In both cases, nitrogen in the raw material was transformed into nitrogen gas (N_2).

Experiment 2: Figure 5 show the relation between COD in the liquid of the reactor output and repetition number when the weight ratio of Ru to Rh is 1 to 1. COD of the raw material and the control are both the same as those in Experiment 1. In any honeycomb catalyst of this experiment, CODs behave alike. The small COD during the first batch test, followed by the sudden increase after that, is similar to the case of the Ru catalyst in Experiment 1. The consistancy of COD after 3 or 4 repetitions is similar to the case of the Rh catalyst in Experiment 1.

Figure 6 shows the changes in the nitrogen quantity in the liquid of the reactor output with repetition. In this figure, the results when Ru/Rh is 1/1 are shown. Contrary to the case in which Ru or Rh was separately supported on a ceramic honeycomb, both Kjeldahl nitrogen and nitrate nitrogen were very small in quantity. Most of the nitrogen in the raw material was detected in the gas as N_2. When Ru/Rh is 1/3, the Kjeldahl nitrogen ranged from 0.3 to 26.9 mg/l and nitrate nitrogen ranged from 112 to 211 mg/l. When Ru/Rh is 3/1, the Kjeldahl nitrogen ranged from 0.3 to 21.8 mg/l and nitrate nitrogen ranged from 246 to 391 mg/l. In any case, the Kjeldahl nitrogen was very small compared to total nitrogen. The larger the ratio of Ru to Rh, the larger the nitrate nitrogen quantity became.

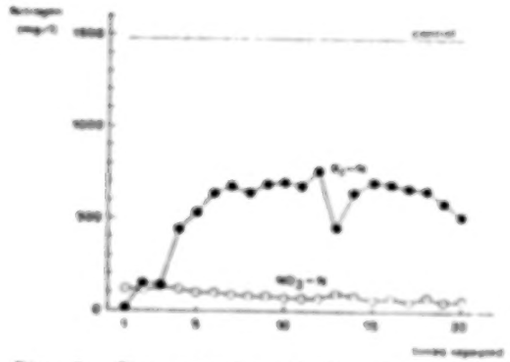

Fig. 3. The effects of the Rh catalyst on the chemical form of nitrogen

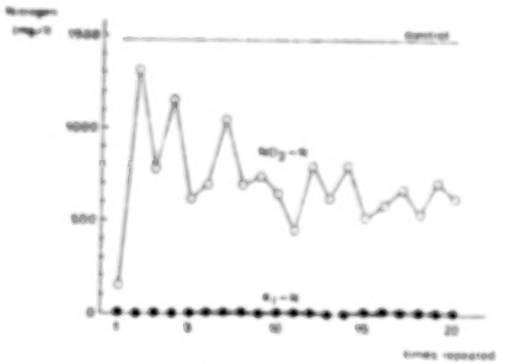

Fig. 4. The effects of the Ru catalyst on the chemical form of nitrogen

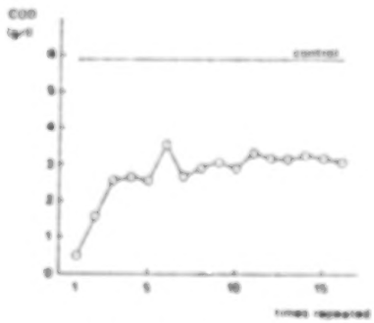

Fig. 5. The effects of Rh+Ru catalyst on the COD reduction (Ru/Rh = 1/1)

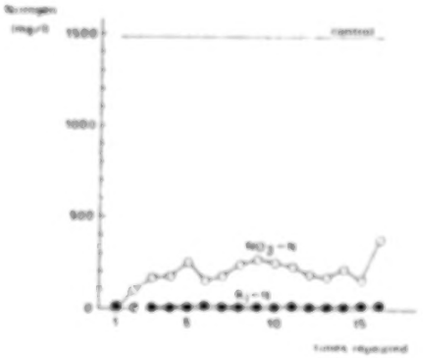

Fig. 6. The effects of Rh+Ru catalyst on the chemical form of nitrogen

Table 1 The Summary of the Experiments

	no catalyst	Rh	Ru	Pd	Pt	Ru+Rh
oxidation	-	+++	+	+	-	+++
nitrogen form	NH_4	NH_4+ N_2	NO_3+ N_2	N_2	N_2	N_2+ NO_3

DISCUSSION

Summary of the experiments : Table 1 shows the summary of the experiments. It was discovered that complete nitrogen gasification as N_2, which will be used as a component of air in a spaceship, is carried out by the use of Pt or Pd honeycomb catalysts, although in each case the COD reduction is low and/or unstable with repetition. A catalyst which would accomplish both nitrogen gasification and complete oxidation could not be found, but a promising one was found (Ru+Rh). If the main nitrogen form needed in CELSS is N_2, then this catalyst is considered to be a good candidate for use in CELSS. COD reduction was higher and more stable than in the case of Pd or Pt. The transformable catalyst of the organic nitrogen to nitrate form, which plants in hydroponic solution utilize the most, could be found (Ru), but the nitrified nitrogen occupied about half of the control nitrogen and the rest was gasified as N_2. In addition, a high rate and stable oxidation was not achieved.

If the wet-oxidation facility for CELSS is made under the present conditions, the two-step wet-oxidation will be adoptable. In the first step, wet-oxidation is carried out without catalyst. The organic substances in the raw material is oxidized to some extent, and the suspended matter which often poisons a catalystis is dissolved. In the second step, wet-oxidation is carried out with catalysts. The organic matter remaining at the first step is oxidized completely and the chemical form of nitrogen is controlled.

In the near future, the chemical form of nitrogen in the reactor output will be fully controlled by the use of noble metal catalysts. The largest problem to be overcome is that oxidation will not occur completely. To solve this problem in a short time span, countermeasures such as an increase in the catalyst quantity, reaction temperature, reaction time etc. will be taken, which will partially solve this difficulty. Generally speaking, the durability of a catalyst mainly depends on the solubilization or the release of catalytic compounds from its carrier and, on the other hand, the property of a catalytic reaction mainly depends on the combination of elements. Therefore, in a long time span, measures such as the exchange of carrier material to silica or titania, the addition of another element to Ru and Rh, the change of the combination of catalytic compounds and so on are to be researched hereafter.

The subjects to be solved: The subjects of wet-oxidation in the application to CELSS are as follows. The first is to find out the catalysts with which both complete oxidation occurs and the chemical form of nitrogen is controllable. The second subject is the correct measurement of the material balance. In addition to carbon and nitrogen, elements to be traced are P, K, Ca, Mg and S, which are macro nutrients of plants, Fe, Mn, B, Cu, Zn, Mo and Cl, which are micro nutrients of plants, and Al, Si, Cr, Ni, Hg, Pb, Cd and As, some of which are harmful to plants or the human body. The third is to examine and evaluate the quantity of the catalyst effused from the wet-oxidation system, though it is thought to be much smaller than in the case of copper compounds. The fourth is the measurement and evalution of aldehyde, alchol, carboxylic acids and other compounds with low molecular weights and low boiling points which are produced in wet-oxidation as end products in addition to acetic acid. If these compounds are produced in significant quantities, then the necessity to prevent these compounds from being produced or to remove them from the liquid or gas will be of importance.

On the other hand, research on making up a practical wet-oxidation facility for CELSS has been delayed. In its construction, it is noticed first that this system will be used under the condition of zero gravity. Problems such as leakage, elusion and wear will result from the high pressure and temperature and a more in-depth study is needed for the solution of these problems. As for the material of the reactor, for instance, the wet-oxidation facility on the earth first used SUS 316, then titanium was gradually adopted. The best material in space is still unknown. Examination of the effectiveness of such new materials as ceramics have yet to be fully tested. The investigation of the system composition of wet-oxidation in CELSS and of the materials and mechanisms to be choosen in each part needs to have a more intensified study carried out on it in the future. This study will be achieved with more effort than that on wet-oxidation reaction itself which the authers have been researching.

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N88-12265

A LARGE-SCALE PERSPECTIVE ON ECOSYSTEMS

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ABSTRACT

Interactions between ecological elements must be better understood in order to construct an ecological life support system in space. An index was devised to describe the complexity of material cyclings within a given ecosystem. It was then applied to the cyclings of bioelements in various systems of material cyclings including the whole Earth and national economies. The results show interesting characteristics of natural and man-made systems.

INTRODUCTION

In order to construct an ecologically based life support system in space, we must first understand the interactions of ecological elements in ecosystems on the Earth. Without knowledge of these interactions, a mere combination of the elements may not result in anything resembling what we originally intended to create. Its necessity becomes more manifest when we try to envision construction of a completely closed, regenerative life support system that can accommodate tens of thousands of people.

Then, do we have the understanding? I am afraid that we do not. We do not know, either in theory or in practice, how the elements in an ecosystem interact. The environmental problems we face today illustrate the inadequacy of the understanding /1/.

Natural ecosystems develop continuously through a process called succession. The culmination of the succession is a stabilized ecosystem in which maximum protection from perturbations is achieved. There, nutrient cyclings are more or less closed, while they are open before reaching the stabilized condition.

Closed nutrient cycling is not very productive /2/. In the past, man has generally been interested in obtaining maximum production from ecosystems by developing and maintaining early successional types of ecosystems. This tendency is deeply rooted in the makeup of our science and society, and is a principal cause for the present environmental problems of anthropogenic origin.

This nature of ecosystems presents a conflict we have to face in constructing a closed, ecological life support system. Because we would like it to have a high production rate for life support materials such as molecular oxygen, food, clean water, and fresh air on one hand, and also would like it to be closed and stable on the other hand. We need to recognize, if we are to be successful in establishing an ecologically based life support system, that we are going to have to deal with a system with which we are not very familiar. Therefore, it is of paramount importance to have a perspective viewpoint on what, materialwise, natural ecosystems do and how man-made systems are different from natural ones.

SYSTEMS OF MATERIAL CYCLINGS, NATURAL AND ARTIFICIAL

In an ecological system there are various elements that transfer, transform, and/or store physicochemical resources such as oxygen, organic compounds, water, and carbon dioxide. In order to put various systems of material cyclings in perspective, these elements are grouped according to their activity. For instance, a pine tree in a natural forest may be regarded as an element that transforms water and carbon dioxide to molecular oxygen and organic matter. Likewise, a factory that burns oil is an element that transforms organic carbon to inorganic carbon. Unlike the tree, the location of the factory and the kind of products it yields are largely determined by reasons unrelated to ecological and biological reasons. However, its very existence certainly affects the carbon and other material cyclings. Details of the classifying method are given elsewhere /3,4/.

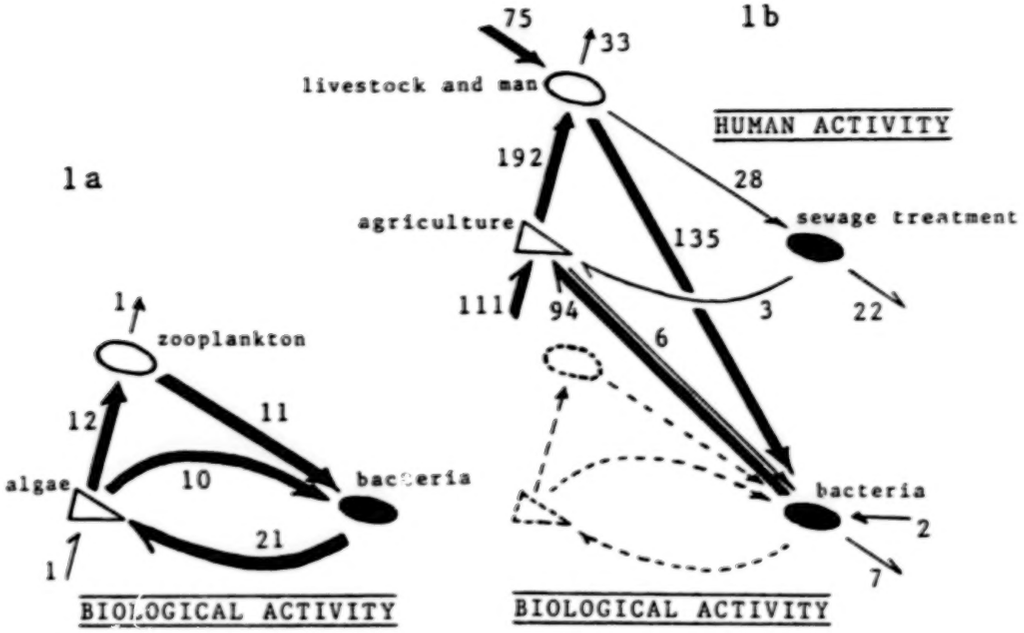

Fig. 1. Natural and man-made phosphorus cycles. Names indicate representative elements that are responsible for the transforming activity. Imbalance between inputs to and outputs from each transforming activity results 1) from the accumulation or the loss of phosphorus from the ecosystem and/or 2) from an incomplete account of the phosphorus budget. Shape of arrowhead distinguishes the kind of phosphorus: ⟶ for a flow of organic phosphorus and → for a flow of inorganic phosphorus. Fig. 1a the cycle in the trophogenic zone of a eutrophic lake: the numbers indicate phosphorus flux relative to the amount of incoming inorganic phosphate; thick arrows stand for a flux 10 times larger than the incoming phosphate; the basis for the figure is from Rigler /5/. Fig. 1b UK phosphorus economy: all numbers refer to thousands of tons P/year; data are based on Bowman /6/; the annual flow of 75 kilotons P or more is indicated by a thick arrow, while that between 33 and 2 kilotons P by a thin arrow; flows less than 2 kilotons P are not shown.

Figure 1 compares the phosphorus cycle in the trophogenic zone of a eutrophic lake (1a) with the phosphorus economy of the United Kingdom (1b). In this particular model eutrophic lake, one unit of inorganic phosphorus enters the ecosystem and the same quantity of phosphorus sediments in the form of zooplankton feces. The amount of phosphorus that moves along with the four paths that constitute the phytocycle is one order of magnitude higher than those incoming and outgoing. The phosphorus cycling in the zone, therefore, can be considered closed.

The extent of the closure is higher in some ecosystems. For example, results compiled by Paul and Voroney for typical natural grasslands show that they have no output of phosphorus and that the accumulation of phosphorus in the soil is 0.05 gP/m^2/year, while the flow of phosphorus is 4.36 gP/m^2/year from inorganic to organic and 4.41 gP/m^2/year from organic to inorganic /7/. This demonstrates not only closure but also a balance in the amount of material flow in natural ecosystems.

In clear contrast with the natural phosphorus cycling, the UK phosphorus economy is open and uneven. Every year, more than 100 kilotons of inorganic phosphorus is imported and used for agriculture, and 75 kilotons of organic phosphorus comes from overseas. Organic phosphorus excreted by domestic animals and humans goes largely to natural, soil microorganisms. Although soil microorganisms return 94 kilotons of phosphorus in the form of inorganic phosphate to agricultural crops, man's heavy reliance on phosphorus mineralization by natural microorganisms might be imposing a stress to natural soil ecosystems. In fact, the phosphorus economy of Japan /8/ shows that 110 kilotons P is the annual load from livestock and humans to natural soil microorganisms and the return to agriculture is 22 kilotons/year. This imbalance must be a cause for the wide-spread eutrophication of waters in Japan.

The closure and the balance of material flows within a natural ecosystem have been also reported for nitrogen cyclings within mature ecosystems such as tallgrass prairie /9/ and northern hardwood forest /10/, while both sociogeochemical cyclings of carbon and of nitrogen exhibit the openness and the unevenness /11/.

AN INDEX OF MATERIAL CYCLING

Earlier, Mizutani and Wada /3/ proposed an index, H_a, to quantitatively analyze the activity of life on the Earth. The index is a modification of Shannon's index of diversity, and the modification was specifically made to illustrate the importance of material flow and conversion in ecosystems. They made a preliminary analysis of global carbon cycling to evaluate the impact of man on the cycling. The earlier index was interested only in flows between groups within the system. However, in an actual ecosystem, inputs to and outputs from it also have a significant influence on the ecosystem. These flows must be especially important in the case of human intervention and of apparently open, natural ecosystems such as rivers, estuaries and rookeries /12,13/.

Therefore, it is proposed to include inputs and outputs in the index. The revised equation for the H-index is:

$$H_a = -\sum_{i=1}^{N}\sum_{j=1}^{N} p(i,j)\log_2 p(i,j) \quad \text{and} \quad p(i,j) = \frac{f(i,j)}{\sum_{i'=1}^{N'}\sum_{j'=1}^{N'} f(i',j')} \tag{1}$$

where a stands for a physicochemical species (or a group of species) of interest, N for the number of groups of elements involved in the activities of transforming species a in an ecosystem, N' for the number of the groups including inflows and outflows, and f(i,j) for the quantity of species a flowing from group i to group j. A path within one group is defined as nonexistent: i.e., f(k,k)=0 for any k.

As N varies depending on each system of material cycling, so does the number of possible paths within it. In order to make a comparison between different systems, the index H_a is divided by the maximum number the index can theoretically take.

The index after this normalization is: $$H_a = \frac{H_a}{H_{max}^{Th}} \tag{2}$$

where H_{max}^{Th} is the maximum and is given by: $$H_{max}^{Th} = \log_2 P \tag{3}$$

where P is the number of paths present in the system.

The normalized index would be zero, when no flows between the groups in the system exist. And it becomes unity, when the material cycling within the system is completely closed and the amount of material flowing through each path is equal.

TABLE 1 Systems of Material Cycling and their H-index

SPECIES	SYSTEM	H_a
CARBON	WHOLE EARTH without human activity	0.92
	with human activity	0.53
PHOSPHORUS	GRASSLAND	0.94
	with grazing	0.88
	DECIDUOUS FOREST	0.95
	EUTROPHIC LAKE	0.96
	ECONOMY IN U.K.	0.63
	ECONOMY IN JAPAN	0.56
NITROGEN	WHOLE EARTH without human activity	0.73
	with human activity	0.61
	HARDWOOD FOREST	0.87
	after deforestation	0.72
	TALLGRASS PRAIRIE	0.99
	with grazing	0.76
	plus fertilizer	0.73
	plus irrigation	0.69
	SWAMP	0.67
	MACARONI PENGUIN ROOKERY	0.15
	KING PENGUIN ROOKERY	0.11
	PADDY FIELD without fertilizer	0.13
	with fertilizer	0.28
	TOKYO BAY without human activity	0.23
	with human activity	0.22

H_a was calculated from various published results.

RESULTS

The normalized H-index makes it possible to compare with one another apparently different systems of material cyclings such as the eutrophic lake and the UK phosphorus economy.

Results of a preliminary analysis of various material cycling systems taken from the literature are shown in Table 1. It is summarized as follows: a) For mature, natural ecosystems, H_a is generally near unity; b) Human interventions to such systems decrease H_a; c) Each species has its own characteristic behavior of H_a; d) H_a of man-made systems is far below unity, probably because man is generally interested only in a portion of the whole material cyclings; e) For a paddy field that is an artificial, productive ecosystem, fertilization increases H_a and crop yield, while, for a natural prairie, human intervention decreases H_a; and f) For intrinsically open, natural ecosystems such as Tokyo Bay and seabird rookeries, H_a is low even without human intervention.

The index, H_a, thus appears to characterize the nature of material cyclings within a system. It is a comprehensive measure for the productivity of a material cycling system, and, therefore, can be used to correlate the diversity of material flows with the stability and the productivity of ecosystems. It may be further applied to the development of an ecologically based life support system in space, and give a way to evaluate its ecological elements and its overall design.

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N88-12266

DESIGN OF AN ELEMENTAL ANALYSIS SYSTEM FOR CELSS RESEARCH

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ABSTRACT

The results of experiments conducted with higher plants in tightly sealed growth chambers provide definite evidence that the physical closure of a chamber has significant effects on many aspects of a plant's biology. One of these effects is seen in the change in rates of uptake, distribution, and re-release of nutrient elements by the plant (mass balance). Experimental data indicates that these rates are different from those recorded for plants grown in open field agriculture, or in open growth chambers. Since higher plants are a crucial component of a CELSS, it is important that the consequences of these rate differences be understood with regard to the growth and yield of the plants. This paper will focus on the description of a system for elemental analysis which can be used to monitor the mass balance of nutrient elements in CELSS experiments. Additionally, data on the uptake of nutrient elements by higher plants grown in a growth chamber will be presented.

INTRODUCTION

As Carl Sprengel pointed out over 100 years ago, the productivity of a plant can be inhibited by the shortage of a single nutrient element ("Liebig's" Law of the Minimum), even when all the other elements are in ample supply. In CELSS applications, where high plant productivity is desirable, it is necessary to monitor and maintain the concentrations of all the nutrient elements to prevent this limitation from occurring. Additionally, because plants may take up more than they require of elements that are in high concentrations (i.e. luxury consumption), with potential effects on nutritional quality, it is important to ensure that excessively high concentrations are not reached. For this presentation, we will concentrate on describing an elemental analysis system developed for CELSS research use.

METHODS

Initially, we evaluated four different methods for the monitoring of nutrient elements in hydroponic/aeroponic plant growth systems. These included atomic absorption/flame photometry (AA/FP), inductively coupled plasma (ICP), continuous flow analysis (CFA) and ion chromatography (IC).

The advantages of IC over the other monitoring techniques include:

1) Low purchase price: Approximately $25,000 for an anion/cation system.

2) Low operating costs: Reagents are inexpensive, and the only elements that require replacement in general use are the columns.

3) Very easy to operate: No extensive training is required to run the IC.

4) Small sample size: Only about 0.1 ml sample is required for analysis.

5) May be automated: Auto-samplers are available now, but if a pressurized nutrient line is available, all that is required is to add a solenoid valve between the IC and the nutrient line.

The first step in development of the nutrient analysis system was characterization of the IC. Figure 1 illustrates a typical chromatogram. Preliminary analysis indicated that using peak height as an indicator of ionic concentration was equal to or better than using integrated peak area. Because peak height is easier to measure and significantly less complicated to obtain via a computerized data acquisition system, we chose to use peak

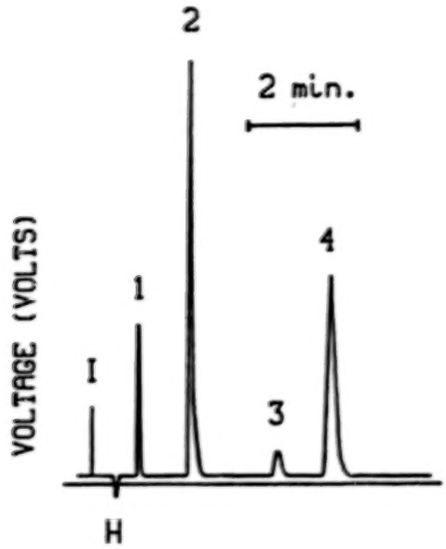

Fig. 1. Typical ion chromatogram. I, injection mark; H, water "peak"; 1, chloride; 2, nitrate; 3, phosphate; 4, sulfate.

height for ionic concentration measurements. Standard solutions were mixed, and standard curves of concentration versus detector voltage were constructed. These standard curves were then used to determine the ionic concentrations in nutrient solution samples collected during a plant growth experiment.

The data presented below was collected during an experiment in which plant growth and uptake of anions from the nutrient solution were monitored. A first crop of lettuce plants were grown in modified, half-strength Hoaglands' solution. For the experiment described below, a second crop of lettuce plants was then grown in the same solution, but supplemented with a solution obtained from wet-oxidation of the lettuce heads from the first crop. This group was compared with a control group grown in a fresh batch of half-strength modified Hoaglands' solution.

The nutriculture system used in the experiment described here is housed in a 3 m^2 controlled environment room, and has been described by Schwartzkopf, et al, 1986. Temperature was 25/20 C day/night, relative humidity was 70%, light averaged 600 $\mu mol/m^2/s$, nutrient solution temperature was 23 C, pH was 6.4, and atmospheric CO_2 was kept at ambient concentration.

RESULTS

Figure 2 is a composite of the standard curves constructed with the IC. Ionic solution concentrations ranged from full strength, modified Hoaglands' to pure, deionized water. As this figure indicates, the IC is a very precise instrument. Standard deviations ranged from 1 to 5% of the value of the mean for each of the four anions.

Figure 3 is a composite showing the concentrations of the anionic species during the course of the experiment. This graph shows the accumulation of chloride in the control and wet-ox solutions. The accumulation of chloride was due to the addition of 0.1 M HCl to maintain pH. The apparent increase in nitrate, sulfate and phosphate during the first two weeks was due to a malfunction in the automated makeup water system, which allowed the nutrient solution volume to decrease by about 20%, thus producing an apparent increase in the concentrations of these three anions. Nitrate uptake was slightly faster in the wet-ox grown plants than in the control plants. Coupled with the lower initial nitrate concentration, this reduced the nitrate level in the wet-ox solution to a barely measurable concentration by the 24th day of the experiment. Phosphate concentrations followed nearly the same pattern in both groups, and final concentrations were equal, despite the fact that the overall concentration of phosphate in the wet-ox solution was about half that of the control. Sulfate, on the other hand, not only followed the same general course of the control, but was equivalent in concentrations as well.

Finally, upon harvesting the lettuce, no statistically significant differences were found between the dry weights of the two groups of plants (Table 1). A blind taste test was conducted with this lettuce, and 40 volunteer subjects found the wet-ox grown lettuce too bitter to eat! We are beginning tissue analysis to determine what caused this bitterness, but it may be due to a nutrient limitation during the last few days of growth, possibly nitrate, as shown in Figure 3 (W. Berry, personal communication). This result emphasizes both the need for nutrient element analysis and the control of mass balance, in order to preserve the nutritional quality of foodstuffs grown in a CELSS.

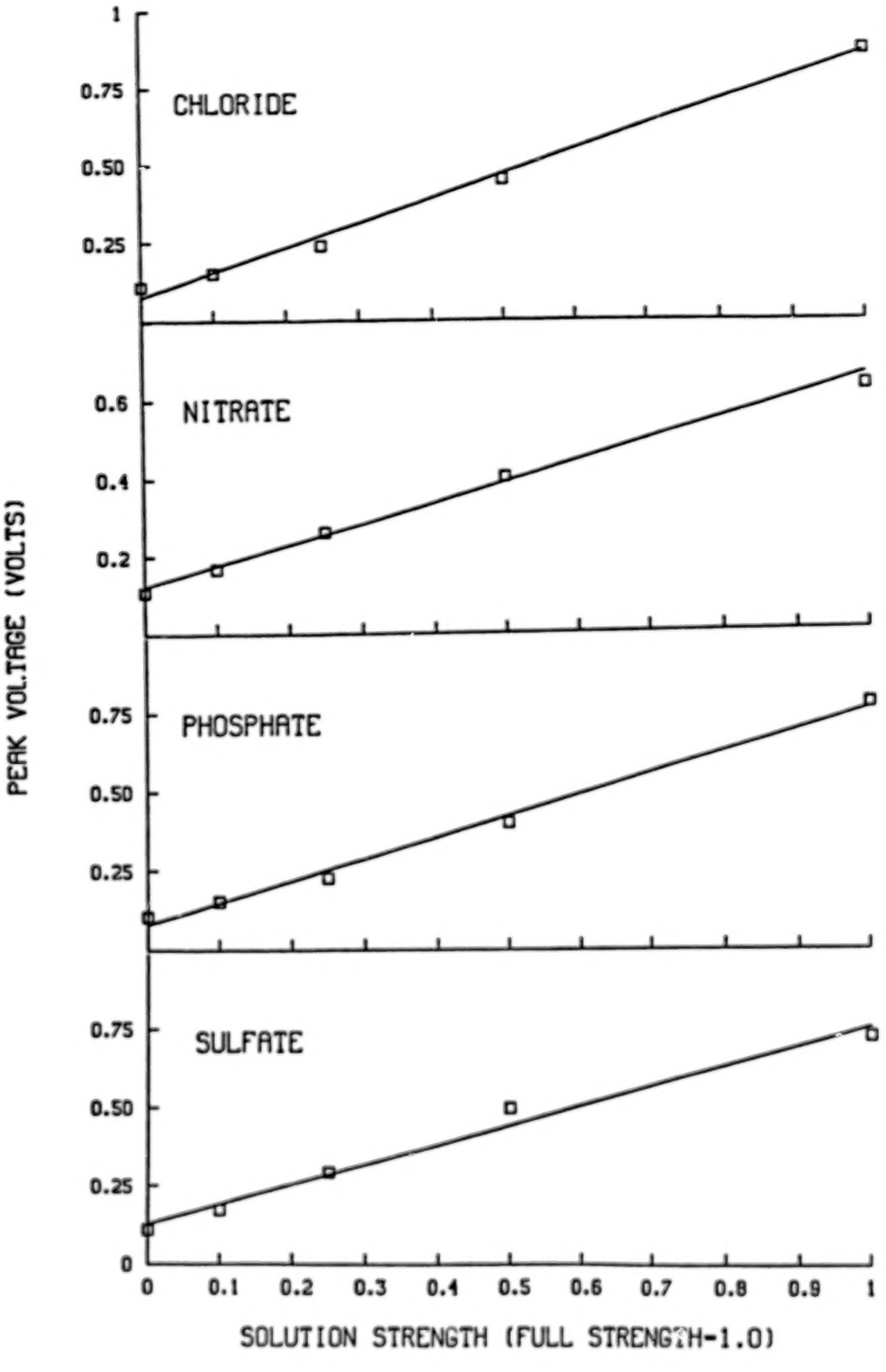

Fig. 2. Standard curves for anions.

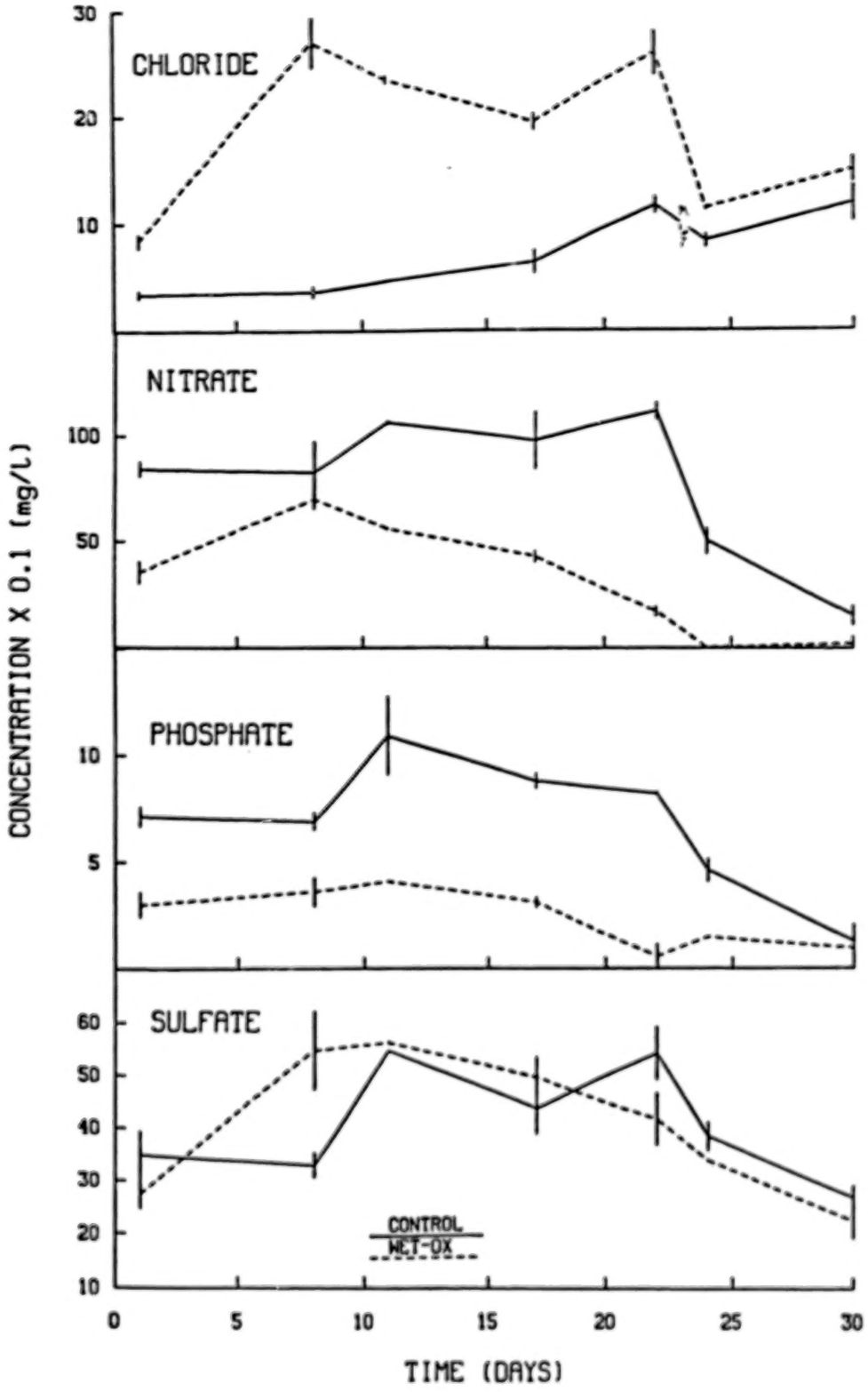

Fig. 3. Anion concentrations during wet oxidation experiment.

TABLE 1 Dry Weights of Tops and Roots of 5 Week Old Lettuce Plants

		Wet-Ox	Control
Top	$\bar{x}$	2.80	2.27
	s	0.80	0.63
Root	$\bar{x}$	0.39	0.48
	s	0.078	0.093

CONCLUSION

The use of ion chromatography for analysis of nutrient solution ion concentrations is an accurate, relatively rapid monitoring method. Initial evaluation indicates that the technique is well suited to the analysis of nutrient solution samples from hydroponic plant growth experiments for CELSS applications.

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N88-12267

II. 3. 1.

An Overview of Japanese CELSS Research Activities

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ABSTRACT

Many research activities regarding Controlled Ecological Life Support System (CELSS) have been conducted and continued all over the world since the 1960's and the concept of CELSS is now changing from Science Fiction to Scientific Reality. Development of CELSS technology is inevitable for future long duration stays of human beings in space, for lunar base construction and for manned mars flight programs. CELSS functions can be divided into two categories , Environment Control and Material Recycling.

Temperature, humidity, total atmospheric pressure and partial pressure of oxygen and carbon dioxide, necessary for all living things, are to be controlled by the environment control function. This function can be performed by technologies already developed and used as the Environment Control Life Support System (ECLSS) of Space Shuttle and Space Station. As for material recycling, matured technologies have not yet been established for fully satisfying the specific metabolic requirements of each living thing including human beings.

Therefore, research activities for establishing CELSS technology should be focused on material recycling technologies using biological systems such as plants and animals and physico-chemical systems, for example, a gas recycling system, a water purifying and recycling system and a waste management system.

Based on these considerations, Japanese research activities have been conducted and will be continued under the tentative guideline of CELSS research activities as shown in documents / 1/,/2/.

The status of the over all activities are discussed in this paper.

INTRODUCTION

The appropriate quantities of oxygen, food and water should be continuously supplied to human beings in order to provide metabolic requirements and all waste materials such as carbon dioxide gas, induced contaminant gas, feces, and urine are to be taken away from their living space to sustain hygienic requirements.

Both the oxygen and food requirements for animals, including human beings, is well known, are originally produced from the photosynthetic reactions of plants and algae.

Suitable conditions for each plant and algae are automatically controlled by the functions of nature itself on the earth, i. e., the carbon dioxide concentration needed for plant growth is regulated by the reservoir function of sea-water, and the necessary quantities of water for all bio-species are supplied through the evaporation of sea-water, atmospheric circulation, rain and the water flow on and under ground. Temperature is automatically controlled by the energy balance between solar energy input and thermal radiation from the earth. Waste materials such as feces and urine of animals and the non-edible parts of plants are decomposed to inorganic components and minerals for fertilizer by the functions of microbes, protozoa and natural oxidation.

In an artificial space like the inside of pressurized modules, natural convections and regulations of materials such as carbon dioxide gas, water and so on can not be expected to occur and a large cultivating area and volume for plant and algae would be required to supply enough food and oxygen gas to human beings, if the plant and algae were cultivated under environmental conditions similar to the natural conditions on the earth.

Under the processes of development and evolution, almost all plants, as is well known, have accumulated a latent ability to adapt to different conditions from the present carbon dioxide concentration on the earth, and it is preferable to introduce a higher carbon dioxide concentration system for cultivating plants and algae efficiently in CELSS.

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In order to maintain different gas conditions for autotrophs (plants & algae) and for heterotrophs (animals & human beings) the introduction of a Gas Recycling System is essential in CELSS.

As for recycling of waste and foods, applicability of the bio-systems to the narrow artificial space has various problems as follows ;
(1) How to reduce methane gas emitted from the waste management system.
(2) How to avoid the leakage of pathogenic bacteria from system.
(3) How to manage the organic sludge after the fermentation is over.

Therefore the physico-chemical system such as wet oxidation waste-manegement system is to be adopted, rather than the bio-system, as the main candidate for CELSS subsystem.

From these considerations. the system configuration of CELSS, as shown in Fig. 1., has been tentatively determined through the conceptual study described in Document /3/.

Almost all Japanese CELSS research activities have been conducted under the consideration of this system configuration in order to determine how to construct and control the total system including bio-systems and physico-chemical systems for establishing stable material recyclings.

Gas Recycling System

To supply an atmosphere of different gas concentrations to the plant cultivating facility, to the animal vivarium or the living quarters of human beings, all exhausted gases from plants, algae, animals and human beings are to be collected and sent into the gas recycling system. In the gas recycling system, each gas (oxygen, carbon dioxide and nitrogen) are to be separated, concentrated and stored in gas containers for mixing and resupply suitable gas concentrations, through gas manifolds, to the plant cultivating facility, to the animal vivarium or to the living quarters of human beings. Therefore, the gas recycling system has to have, at least, the following functions; carbon dioxide separation and concentration, oxygen gas separation and concentration and gas content regulation and re-supply /4/.

As for the separation and concentration of carbon dioxide gas, three types of the carbon dioxide absorption and desorption system have been developed and tested for application to the environment control system of the Japanese Experiment Module (JEM), to be attached to the Space Station.

Two systems using different kinds of the absorption and desorption agents, one agent is composed of micro porous beads coated with polyethylene-imine and another is composed of an ion exchange resin with an amine base, have been tested to evaluate the performance characteristics of each system developed independenty by Kawasaki Heavy Industries, Ltd. (KHI) /5/ and Mitsubishi Heavy Industries, Ltd. (MHI). Sumitomo Heavy Industries has conducted their own experiments to compare the performance characteristics of two systems using the solid amine (ion exchange resin) and using a molecular sieve /7/. Mitsubishi Electric Corp. (MELCO) has proposed a new idea for the absorption and desorption of carbon dioxide using a super cooled molecular sieve and is now conducting a conceptual system design study using this new idea /8/.

On the other hand, Professor Nakabayashi of Tokyo Medical & Dental University, with the assistance of Fujikura Kasei Co. Ltd., has conducted research for obtaining an excellent, and more suitable, absorption and desorption agent by a copolymerization method between macro porous beads and various kinds of amines /9/. Table 1. shows the carbon dioxide absorption characteristics of various agents developed through his research activities.

As mentioned above, various intensive studies regarding the carbon dioxide separation and concentration have been conducted during the past two years by individual companies under the guideline of our CELSS research activities. The necessary technologies for a carbon dioxide separation and concentration system seem to be almost established and should be completed in the near future.

As for oxygen gas separation and concentration, the system design study and experimental test for developing an oxygen separation and concentration system using salcomine agent have been conducted by Kawasaki Heavy Industries under contract to the National Aerospace Laboratory, Fig. 2., /10/.

Fig. 3. shows the canister to be used for the carbon dioxide or oxygen concentration. This type of canister has distinctive features, for example, the heating and cooling, required for desorption and absorption, can be easily done because of the large surface area of gas contact. Additionally, there is an absorbent holder mechanism associated with the canister.

Based on these study results the integrated design studies for obtaining gas recycling system are under way in the National Aerospace Laboratory /11/.

Gas exchange functions between heterotrophic and autotrophic bio-species will be established under the support of this gas recycling system in CELSS.

Excess quantities of oxygen or carbon dioxide, due to the imbalance between the assimilation quotient and respiration quotient, may be generated in the total loop of gas recycling. Excess

oxygen can be consumed easily and quickly by oxidizing the carbon in this loop. Otherwise, the generation of excess carbon dioxide gas becomes a serious problem for keeping a stable ecology because the excess quantity of carbon dioxide can not be quickly transfered to oxygen using the photosynthetic reaction of plants and algae.

Therefore, subsidiary oxygen recovery systems using physico-chemical methods such as Sabatier reaction and / or Bosch reaction are to be developed for emergency use. As for Sabatier and Bosch reactors, fundamental experiments about reaction mechanisms and system design studies are now conducted by MHI / 12 /, Fig. 4

Water Recycling System

According to the metabolic requirements of every bio-species, the appropriate quantity of high quality drinking water should be supplied to sustain the life of the bio-species. In addition to such metabolic requirements, hygiene requirements for human beings are also neccessary. Generally, the hygiene water quality requirements, such as COD or BOD etc. are not as strict as the metabolic requirements.

Therefore, the system should be composed of two main loops, the filtration recycling loop and the phase change purification loop. The filtration recycling loop is to be assembled using the permeate membranes such as ultra fine and reverse osmosis filter membranes. Permeabilities of membranes depend on the material characteristics of the membranes themselves.

Professor Ohya of Yokohama National University has conducted research to find the preferable membrane materials for the ultra and reverse osmosis filtrations of waste water, in order to assist the design of the filtration recycling loop.

Hitachi Ltd. and Sasakura Engineering Co. Ltd. have also worked on designing and testing the water recycling subsystems under contracts to the National Aerospace Laboratory / 13 /, / 14 /.

As for the filtration recycling loop, a Bread Board Model, as shown in Fig. 5., has already been developed and evaluated. As for the phase change purification loop, a Bread Board Model of a VCD (Vapor Compression Distillation Unit), as shown in Fig. 6., has also been developed and the experimental tests for finding optimum driving points are now under way in the National Aerospace Laboratory and the Sasakura Engineering Co. Ltd. Another type of phase change purification method, which uses hydrophobic porous membranes to evaporate the water, called the thermopervaporation method, has been studied experimentally by Hitachi, Ltd / 15 /.

Waste Management System

The wet oxidation method for decomposing waste and non-edible parts of plants and animals is one of the most preferable candidates for the CELSS waste management system.

However, low - grade carboxylic acids, such as acetic acid, remain, in the residual liquid after the oxidation process is terminated.

In order to avoid the generation of carboxylic acid, catalysts such as ruthenium are essential to be introduced in the reactor. The problem is how to get stable catalysts for long - term operation. Dr. Takahashi, Niigata University, in corporation with the National Aerospace Laboratory, has conducted research for elucidating the oxidation mechanism and for obtaining more stable catalysts, / 16 /, /17 /. In addition to this study, Dr. Watanabe, National Institute for Environmental Studies, has proposed another method for eliminating carboxylic acid using photosynthetic bacteria, / 18 /.

Plant and Algae Physiology and Cultivation Technologies

To realize the economic cultivation of plants and algae in space, it is necessary to deteimine the latent physiological abilities of plants and algae, and to develop a more economical nutrient solution supply system. Dr. Inada and Mr. Takanashi, National Institute of Agro-Resources, and Dr. Takatsuji and Dr. Kaneko, Hitachi, Ltd. are now studying periodical lighting effects on the photosynthesis of plants such as rice, mug, bean, komatsuna, lettuce and pimento to obtain data concerning gas exchange, growth rate, and harvest index, using their own phytotron as shown in Fig. 7., / 19 /, / 20 /, / 21 /.

Dr. Ohmasa, National Institute for Environmental Studies, and Professor Nishi, Science University of Tokyo, are now studying the information system of pathogenical diagnosis and an instrument system for determining the quantities of exhausted oxygen and assimilated carbon dioxide., / 23 /, / 24 /.

As for algae, Dr. Matsumoto and Professor Ohya, Yokohama National University, Dr. Nagamune and Dr. Endo, The Institute of Physical and Chemical Research, and Mr. Oguchi and his colleagues at the National Aerospace Laboratory are now studying a gas exchange module composed of porous membranes, to be used for algae cultivation, automatic cultivation methods and the total integration method for developing an algae cultivator for space-use. / 25 /, / 26 /, / 27 /.

Animal and Fish Physiology and Breeding Technologies

Animal and Fish Breeding becomes essential for supplying animal protein to human beings in space. Animals and fish have the same hetetrophic characteristics as human beings and thus consume oxygen and exhaust carbon dioxide. The waste of animals and fish generate ammonia and harmful gases such as indole, skatole etc. Therefore, the gas exchange characteristics of each animal and fish are to be accurately determined to balance the oxygen and carbon dioxide gas exchange between the autotrophic and the heterotrophic bio-species and the harmful gases are to be removed and reduced.

Dr. Muramatsu, National Institute of Animal Industries, and Professor Nishi, Science University of Tokyo are studying the instrumentation system for acquiring the metabolic gas exchange data /23/, /28/. Mr. Sudo of KHI is now studying how to eliminate the ammonia gas from the vivarium. Dr. Tamura and Dr. Suzuki, National Food Research Institute, are now investigating the nutrient values of each plant to the animals and the human beings for determining what kinds of plants are desireable in space /29/.

In addition, Mr. Oguchi, National Aerospace Laboratory, is now developing and testing a closed type of aquarium for demonstrating semi-closed ecology experiments, with the colaboration of Mitsubishi Rayon Co, Ltd., as shown in Fig. 8.

Gravitational Effects on Plant and Animal

Success of long run plant cultivation experiments conducted by the Soviet Union has been frequently reported. Unfortunately, detailed data about the effects of micro-gravity on the physiology of plants are scarcely presented.

In the early stage of CELSS experiments, micro gravity facilities, such as the Space Station module, would be useful for verifying the CELSS concept, because artificial gravity facilities such as a lunar base or space colony will not be developed until around 2010 to 2050. Therefore, gravitational effects on the physiology and morphology of plants and animals should be studied onboard Shuttle or Space Station.

Professor Ito, Nagoya University, Dr. Yamashita, Institute of Space and Astronautical Science, and Professor Yatazawa, Nagoya Institute of Technology, are now conducting research on the gravitational effects on plant growth using the Biaxial Clinostat as shown in Fig. 9., /30/.
Their studies are now being concentrated to:
(a) polarization of the axial organs (stem, root),
(b) differentiation and development of tissues and organs,
(c) morphogenesis of an evidently specific form of organs,
(d) interference of gravity with cell division and cell development.

Dr. Ishikawa, Kyorin University, has studied the gravitational effect on the geotropic phenomena by measuring the electric potential of cell membranes in the elongating part of bean roots. Dr. Mizutani and his colleagues, Mitsubishi-Kasei Institute of Life Science, are conducting basic research about the gravitational effects on development and morphogenesis of various bio-species's organs. /31/, /32/.

Bio-reactor in CELSS

Food production using plant photosynthesis is not always efficient and economical because the reaction rate in organs is restricted by various factors and because every bio-species have inherent unstable characteristics. In order to overcome such inconveniences, it seems better to adopt the bio-reactor for producing foods from carbon dioxide in atmosphere instead of the utilization of plant. Professor Ohshima, Tokyo Institute of Technology, has been investigating how to construct the bio-reactor for fixing carbon dioxide and producing food using various enzymes, /33/.

Future Study Plan

As already mentioned, almost all Japanese CELSS research activities have been conducted along the guidelines produced from concept study of CELSS /1/.
However, this guide line is tentative because detailed information about the technical problems could not be obtained at the initiation of this concept study. Therefore, this guide line should be revised based upon the results of research activities now being conducted. This revision study is to be started in the very near future, perhaps at the end of this year, and continuing for two years. In parallel with this study the research activities for solving each technical problem, in order to develop the equipment and facilities needed for conducting the closed recycling test, should be performed and continued. After this study, ground based closed experiments for evaluating the control strategy of CELSS are to be conducted during two or three years. Tentative study plans are shown in Fig. 10. The progress of these plans would be affected by the amount of government investment.

Conclusion

The over all images of Japanese CELSS activities now being conducted and the sketch of next research plans have been briefly reviewed and discussed. The important thing to do now is to get adequate research funds and to expand the research community.

Multilateral and / or Bilateral International Corporations seem to be very useful for accelerating research investment and for expanding the community.

Therefore, the initiation of mutual communications for promoting international corporation seems to be very important and preferable.

The author wishes to acknowledge the support of the members of the Japanese CELSS research group and their technical information exchanges.

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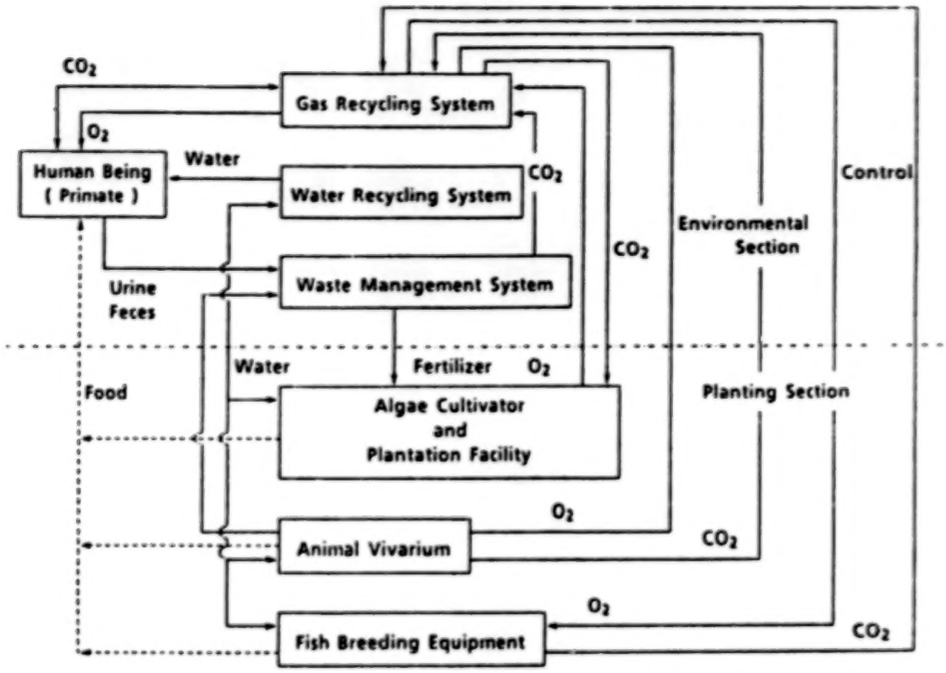

Fig. 1. System Configulation of CELSS

Table 1. CO_2 saturated adsorption amount of CO_2 adsorbent containing various amine compounds

Amine compound	Mw	Solvent	CO_2 adsorbed
Diethy lenetriamine	103	Water / Ethanol	1.68 (Wt %)
Polyethyoeneimine	600	"	2.28
"	10000	"	2.70
"	70000	"	3.35
Polyallylamined	10000	Ethanol	2.23
"	60000	"	2.55
Diethylenetriamine	103	None	6.42
Polyethyleneimine	300	"	6.85
"	600	"	7.08
"	10000	"	7.05

Macroporus polymer beads : DVB / GMA = 50 / 50Wt%

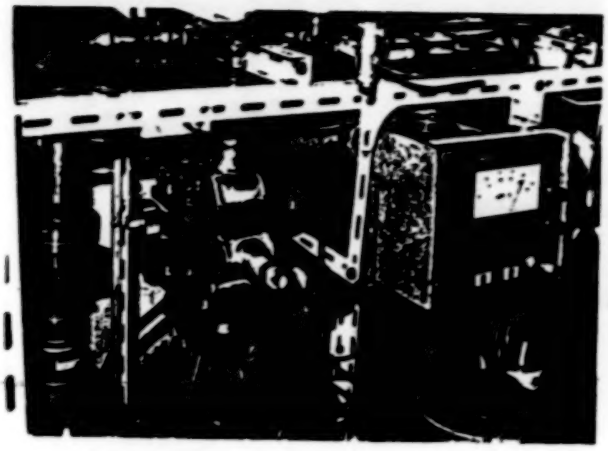

Fig. 2. Oxygen Separation and Concentration System (NAL, KHI)

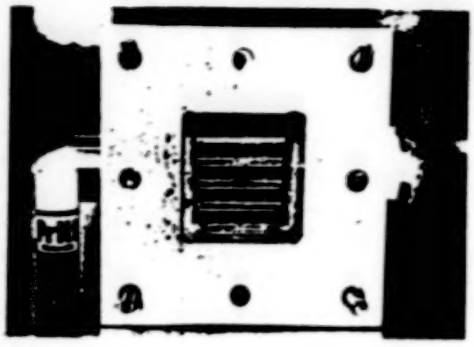

Fig. 3. Canister Used for Gas Separation and Concentration (NAL)

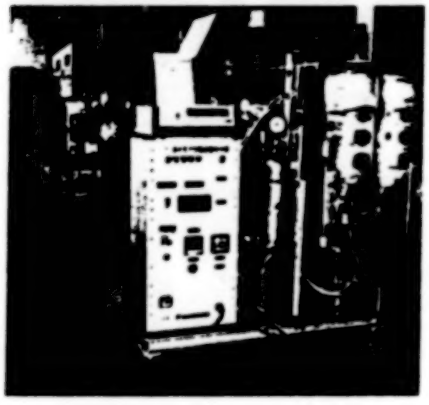

Fig. 4. Bosch and Sabatier Reactors (MHI)

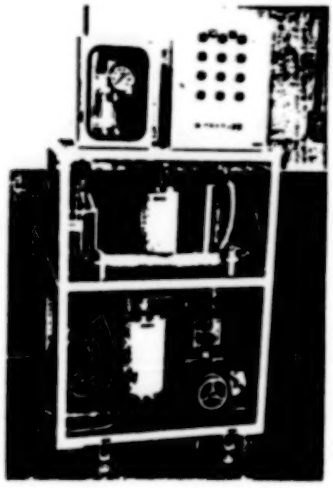

Fig. 5. Water Recycling System Using Filtration Membranes (NAL, HITACHI)

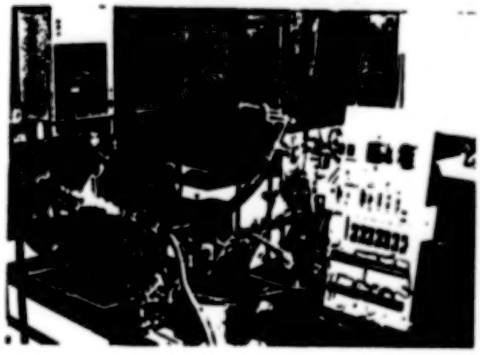

Fig. 6. VCD (Vapor Compression Distillation) (NAL, SASAKURA)

Fig. 7. Test Phytotron (National Inst. of Agro-Resources)

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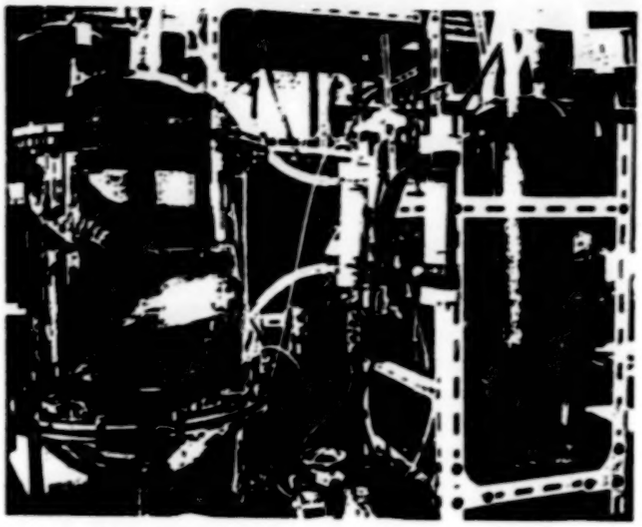

Fig. 8. Semi-Closed Ecology Experiment (NAL)

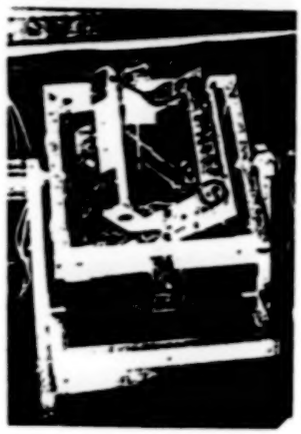

Fig. 9. Biaxial Clinostat (ISAS)

Items \ FY	1987	88	89	90	91	92	93	94	95	96	97
Revision Studies for Future RFP	1st Revision →				2nd Revision →				← RFP →		
IOC Hardware Studies (Phytotron, Algae Cultivator)	Technology Development Study for Hardware →		Phase A/B Studies →		Phase C/D Studies →		Integration & Lunch →		Experiments in Orbit →		
Technology Studies for Closed Test	Technology Development Studies for Each Hardware to be Used in GB CELSS →										
Ground-Based CELSS Experiments							Ground Based Experiment Test →				

Fig. 10. Future Study Plan

N88-12268

ETUDE DES RELATIONS ENTRE PHOTOSYNTHESE RESPIRATION TRANSPIRATION ET NUTRITION MINERALE CHEZ LE BLE

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RESUME

La croissance du Blé *Triticum aestivum* a été étudiée en environnement contrôlé et fermé pendant une période de 70 jours. Les échanges gazeux (Photosynthèse, Respiration) hydriques (Transpiration) et la consommation en éléments minéraux (Azote, Phosphore, Potassium) ont été mesurés en continu. On présentera les relations dynamiques observées entre les différentes fonctions physiologiques, d'une part sous l'influence de la croissance et d'autre part en réponse à des modifications de l'environnement. L'influence de la teneur en CO_2 pendant la croissance (teneur normale ou doublée) sera mise en évidence.

INTRODUCTION

L'étude chez les plantes supérieures des relations quantitatives et cinétiques entre les grandes fonctions physiologiques comme l'assimilation de CO_2, la respiration, la transpiration et l'absorption des éléments minéraux sont très rarement étudiées simultanément. La principale raison en est la complexité (et le coût) des équipements nécessaires qui contraint en pratique à des expériences sur plantules ou organes (feuilles) ou bien à étudier les relations par couple de fonctions par exemple Photosynthèse/Transpiration ; Photosynthèse/Respiration) : plus rarement Photosynthèse/Nutrition. La dispersion des résultats obtenus sur des plantes et dans des conditions différentes rend problématique l'intégration des connaissances sur les plantes supérieures adultes, en particulier dans les régulations entre parties aériennes et racines.

Par exemple la question reste pour une large part posée de l'effet de stimulation de la croissance par le CO_2 sur les activités de nutrition minérale /1/. De même le rôle de la régulation stomatique dans les relations entre Photosynthèse et Transpiration est l'objet de controverses entre un laboratoire américain /2/ et une équipe australienne /3/. Cette dernière observe, dans des expériences sur feuille, que la régulation stomatique est asservie à la photosynthèse plutôt qu'à un état physique interne ou externe, ce qui conduit à une proportionnalité entre Photosynthèse et conductance stomatique ou encore en condition climatique constante entre Photosynthèse et Transpiration. Ceci est observé sur feuille même en cas de limitation hydrique /14/ ; ce résultat est paradoxal, car en contradiction avec l'effet bien connu d'une amélioration de l'efficience de l'eau en cas de limitation hydrique, vérifiée récemment sur le blé dans le laboratoire /5, 6/.

Quoiqu'il en soit des connaissances de base dans ces domaines, l'étude des relations physiologiques entre les fonctions évoquées plus haut devient une nécessité pratique pour prévoir et réaliser la culture des végétaux en cycle écologique fermé du programme CELSS (Controlled Ecological Life Support System) intégré dans les recherchesspatiales. Par la culture de plantes il devra permettre de lever les limitations de distance ou de durée des futures explorations spatiales en produisant une part croissante de nourriture et d'oxygène nécessaire à la vie de l'équipage /7/.

Notre recherche rejoint les préoccupations de ce programme et nous présentons ici l'étude des relations fonctionnelles entre grandes activités physiologiques en régime stable ou perturbé comme base d'une recherche ultérieure sur le rôle de la photorespiration. Le paramètre variable dans cette étude est principalement la concentration en CO_2 connue pour stimuler la photosynthèse mais aussi, et parallèlement, réprimer la photorespiration.

MATERIEL ET METHODES

Les expériences sont réalisées dans le système $C_2$3A précédemment décrit /8,9/.

Procédure de plantation. Après 12 heures d'imbibition dans l'eau, nous avons mis à germer des graines de blé (*Triticum aestivum L.* var. Capitole) dans les conditions de notre laboratoire (température environ 20°C), entre des feuilles de papier filtre. Nous avions calibré les graines au préalable avec le calibreur de semences décrit par Silvy /10/. Les germinations n'ont pas subi de vernalisation. Les plantes sont

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donc restées dans la phase végétative pendant toute la durée des expériences, la variété choisie étant de plus très peu alternative. Nous avons repiqué des plantules de 3 jours dans des pots Riviéra de 1,45 litres (11 x 11 x 12 cm) : 1 plant par pot pour la faible densité utilisée ici. Nous avons choisi de faire ce repiquage afin d'homogénéiser la population au départ. Comme substrat nous avons utilisé du sable qui rendait possible des mesures d'absorption minérale dont le protocole sera précisé ultérieurement. Afin d'éviter au maximum l'évaporation et le développement d'algues, les pots sont recouverts de plastique noir.

Conditions climatiques. Nous avons placé les pots séparés comme décrit dans le paragraphe précédent dans deux cellules jumelles (surface utile de 0,5 m^2 par chambre) à raison de 20 pots par chambre. La concentration en CO_2 est régulée à 330 $\pm$ 5 $\mu l\ l^{-1}$ dans la chambre témoin et à 660 $\pm$ 5 $\mu l\ l^{-1}$ dans la chambre de traitement. Le reste des conditions climatiques est commun aux deux cellules.

Trois lampes Osram HQI-T 400 W/DW assurent un éclairement moyen de 600 $\pm$ 90 $\mu E\ S^{-1}\ m^{-2}$ à une hauteur de 30 cm. Nous avons obtenu une bonne homogénéité d'éclairement entre les deux chambres. Pour la température et la ventilation ce même type de conditions a été réalisé précédemment mettant en évidence une similitude satisfaisante entre les deux chambres. Nous avons élaboré de nouvelles cartes pour l'éclairement pour tenir compte et corriger l'effet de l'usure des lampes. Nous avons utilisé une photopériode de 14 heures de lumière et de 10 heures d'obscurité. La température est réglé à 24°C pendant le jour et 18°C pendant la nuit. Le refroidisseur (Cf. figure 1) est maintenu à une température de point de rosée de 13°C pendant le jour et de 16°C pendant la nuit imposant respectivement une humidité relative de 50 et 85%.

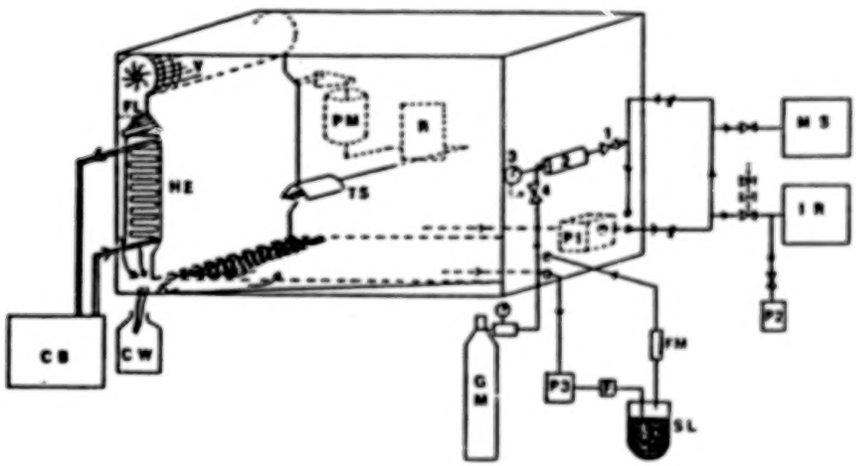


Figure 1
Régulations de température et de la teneur en CO_2 des chambres $C_3$3A

sur la gauche de la figure : régulation de température
TS : sonde de température
R : régulateur à moteur pneumatique
PM : moteur pneumatique
V : ventilateur
FL : volet séparant l'air qui contourne de celui qui traverse le refroidisseur
HE : échangeur de chaleur
CB : bain de refroidissement maintenant l'échangeur de chaleur à la température du point de rosée
CW : eau de condensation

sur la droite de la figure : régulation de la teneur en CO_2
P1 : pompe initiant la cirulation d'air dans le circuit d'analyse
IR : analyseur de CO_2 à infra-rouge (type Hartmann et Braun URAS 2T)
P2 : pompe à vide nettoyant la chambre d'analyse de IR entre chaque mesure. Ceci est nécessaire puisque l'analyseur est utilisé alternativement pour les atmosphères de différentes chambres $C_3$3A.
MS : spectromètre de masse
1 : électrovanne d'injection de CO_2 dans le circuit de retour
2 : réservoir tampon
3 : régulateur de pression dans 2
4 : électrovanne d'injection de CO_2 dans le réservoir tampon
GM : bouteille d'injection contenant un mélange de 20% CO_2 et 80% N_2
P3 : pompe de piégeage de CO_2
F : filtre à charbon actif
SL : piège à CO_2 contenant de la chaux sodée
FM : débit-mètre

Arrosage. L'arrosage journalier dépasse largement les besoins de la transpiration, généralement d'un facteur 2. L'arrosage est répartit en 2, 3 ou 4 fois par 24 h. Il est réalisé par un système volumétrique précis qui distribue une quantité connue de solution dans chaque pot. Nous utilisons la solution nutritive Hoagland n°2 /11/.

Transpiration. L'eau de condensation au niveau de l'échangeur de chaleur (Cf. figure 1) est en réalité l'évapotranspiration du couvert. Elle est pesée quotidiennement avant le début de la photopériode. Pour obtenir la transpiration nous avons corrigé cette quantité de l'évaporation des pots déterminée au début de l'expérience. Bien que cette valeur ait pu varier au cours de l'expérience, l'erreur faite sur la transpiration diminue au fur et à mesure que les échanges de vapeur d'eau augmentent.

Mesures des échanges gazeux. La mesure de la concentration de CO_2 et sa régulation est réalisée à partir d'un analyseur unique et d'un système de mesure et régulation centralisé /9, 12/. La consommation de CO_2 par photosynthèse et son dégagement par respiration nocturne sont déduites des opérations de compensation de ces phénomènes : injections calibrées de CO_2 pendant le jour, piégeage de CO_2 pendant la nuit. Un ordinateur (Télémécanique T1600) anticipe la compensation des échanges gazeux entre deux mesures consécutives faites toutes les 10 mn. Les données d'échanges gazeux et les paramètres physiques sont stockés sur un disque magnétique et moyennés chaque heure, chaque jour et chaque nuit /9, 13/.
Les courbes de croissance sont obtenues en additionnant les bilans journaliers, 14P-10R, où P et R sont les vitesses moyennes de Photosynthèse et Respiration.

Absorption minérale. La méthode est basée sur le lessivage journalier des pots par le haut avec la solution nutritive neuve (quantité connue d'éléments minéraux) et la récupération par le bas de la solution ayant été au contact des plantes. La solution récupérée est pesée quotidiennement et à partir d'une quantité aliquote sont déterminées les concentrations en NO_3, NH_4, PO_4 et K . Par différence entre la quantité d'éléments apportés et récupérés, nous devrions donc obtenir l'absorption minérale par les plantes. Cependant, ceci n'est valable que lorsque la réserve du pot est négligeable par rapport au volume d'arrosage, condition non réalisée dans nos expériences. Nous devons donc tenir compte du volume du pot, ou plus exactement de la variation du contenu en éléments minéraux dans le pot d'un jour à l'autre.
Tous les dosages sont réalisés avec un analyseur automatique (Technicon, 95330 Domont, France). L'appareil comprend un distributeur d'échantillons canaux pour cinq analyses simultanées. Il est piloté par ordinateur /14, 15/.

Méthode des chambres de culture jumelles. Pour renforcer les possibilités de comparaison quantitative entre deux traitements (ici le CO_2) les deux chambres de culture sont alimentées par un même circuit électrique, une même distribution de CO_2, un même système de production d'eau froide et d'alimentation en solution nutritive - la régulation de CO_2 (comme pour la plupart des chambres de culture du laboratoire) utilise un analyseur de CO_2 unique - les sécurités (débit d'eau, température défaut de CO_2 ou de lumière) ont des conséquences symétriques sur les deux lots de plantes et accroît la signification des comparaisons entre elles.

RESULTATS ET DISCUSSION

1 - Profil des échanges au cours du temps

Photosynthèse. La figure 2 présente le profil des vitesses de Photosynthèse nette en CO_2 pendant la phase lumineuse concentration en CO_2. La densité des plantes était faible et leur croissance n'a été limitée par la compétition entre plantes que très tard. Le plateau de Photosynthèse est obtenu vers 50 jours alors que pour une densité 5 fois plus forte il était établi vers 30 jours /5, 6/. Pour des deux types de densité l'indice foliaire était comparable à ce stade et voisin de 2. A la fin de la culture (75j) il était de l'ordre de 10.
Dans la période de Photosynthèse en plateau, qui est typique du fonctionnement d'un couvert fermé, on remarque deux perturbations (jours 48 et 59) causées par de brèves limitations hydriques qui seront analysées plus loin. D'une façon générale les variations, même faibles, sont très bien corrélées entre les deux courbes. Les variations brusques (jours 35 et 38) résultent des variations de densité consécutives aux prélèvements. La compétition entre plantes se faisait déjà sentir puisque la photosynthèse, que l'on a exprimée par plant, est légèrement accélérée.

Respiration nocturne. La figure 3 donne la vitesse de respiration. Les profils suivent ceux de la photosynthèse avec une différence. La respiration continue à croître alors que la photosynthèse est en plateau. Les variations sont bien corrélées entre les deux lots et avec la photosynthèse mais dans ce cas avec de plus faibles amplitudes.

Transpiration. Les mesures de Transpiration (Fig. 4) résultent des mesures journalières des condensats et sont relativement moins régulières. Ceci est dû principalement au fait que, pour ces expériences, les relevés ne sont pas encore automatisés et nécessitaient une opération manuelle de pesée. Elles ne pouvaient pas toujours être faites ponctuellement à la fin d'un nyctémère et si la pesée était retardée la transpiration du jour écoulé était majorée au détriment de celle du jour courant. On pourrait aussi noter l'incidence des jours de fin de semaine pour lesquels une même moyenne est affectée à 3 jours consécutifs. Il reste une bonne correspondance entre traitements et avec la phosynthèse.

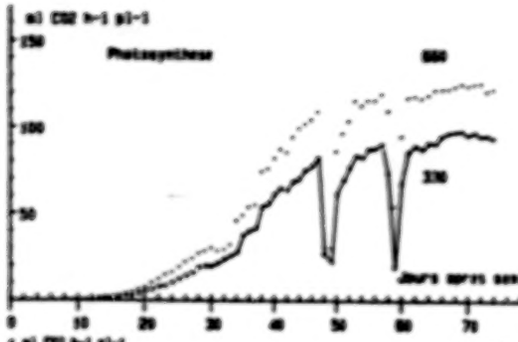


Figure 2 - Vitesse de photosynthèse mesurée sur deux cultures de blé en phase végétative, sous des concentrations de CO_2 de 330 et 660 µl l^{-1}.

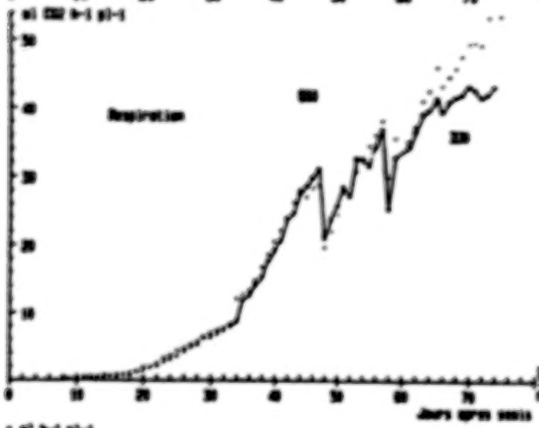


Figure 3 - Vitesse de respiration nocturne mesurée sur les deux cultures de blé.

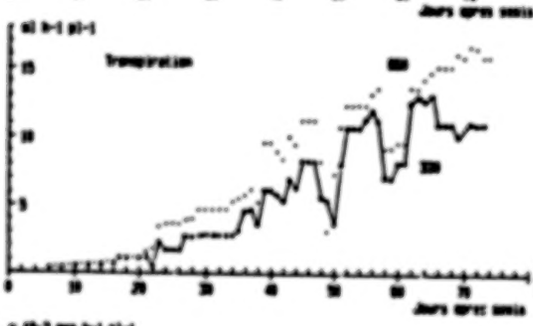


Figure 4 - Vitesse de transpiration mesurée sur les deux cultures de blé.

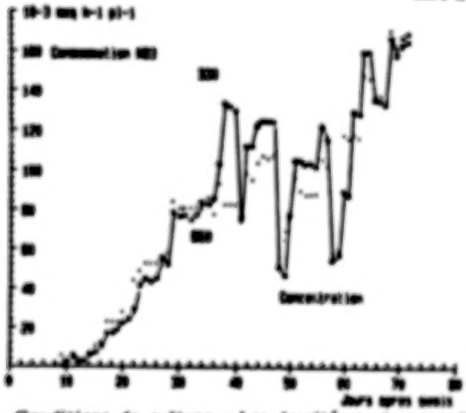


Figure 5 - Vitesse d'absorption de NO_3 pour les deux cultures de blé.

Conditions de culture : Les densités sont respectivement de 40 plants m^{-2} au semis, de 22 plants m^{-2} au 35ème jour et 14 plants m^{-2} au 38ème. L'indice foliaire des deux couverts était respectivement de 1,5 et 1,8 au 39ème jour et de 5,4 et 10,3 au 75ème jour pour les deux couverts.

Nutrition minérale. Les bilans nutritifs journaliers de NO_3, NH_4, K et PO_4 ont été mesurés mais seule la courbe de NO_3 a été représentée Fig.5 pour les deux traitements. Le résultat le plus frappant est l'égalité pratique des consommations alors que les photosynthèses sont très différentes. Un même résultat a été obtenu pour une culture à forte densité /6/.
Une limitation expérimentale ne peut être évoquée pour expliquer ce résultat I) L'alimentation en solution nutritive est systématiquement de l'ordre de deux fois la transpiration, II) La solution percolant dans le sable et recueillie pour analyses n'est épuisée que d'environ 80 % et offre une concentration minimum de l'ordre de 2 mM qui est loin d'être limitante.

Ce type de résultat est transposable aux éléments K et PO_4 évoqués plus loin - le cas de NH_4 est particulier -sauf dans la partie initiale, avant le jour 15, la consommation de NH_4 est totale et sa consommation journalière suit strictement le profil des apports de solution. Il n'y a donc pas de signification physiologique. Ce fait avait déjà été observé chez le maïs /15/.

II - Croissance en biomasse carbonée

La reconstitution des courbes de croissance à partir des bilans journaliers de CO_2 a déjà été étudiée dans la première phase de croissance, jusqu'au 35ème jour, en comparaison avec la culture à forte densité /15, 6/. La figure 6 présente les résultats obtenus jusqu'au jour 70 incluant une longue phase de croissance en couvert fermé. Nous insisterons ici sur la précision et la richesse des informations que l'on peut obtenir par la maîtrise des échanges de CO_2 en chambres jumelles. (Fig.6)

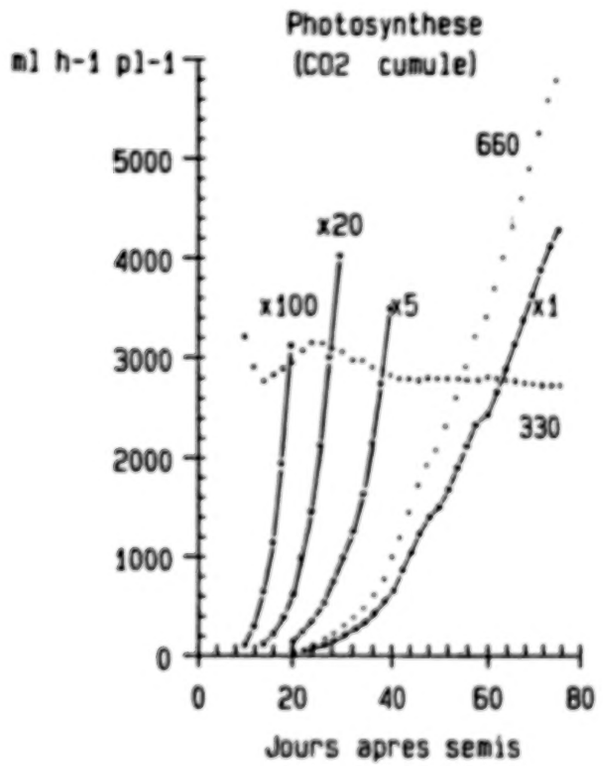


Figure 6 - Courbe de croissance exprimée en cumul de CO_2 assimilé, pour les deux cultures sous 330 et 660 µl l^{-1}. On note la grande dynamique de mesure de croissance (de 1 à 100) et la constance du rapport entre les deux courbes (•) qui est compris entre 1,4 et 1,6. Entre les jours 42 et 64 la moyenne des valeurs journalières de ce rapport est de 1,392 ± 0,005.

On observe la grande dynamique des mesures qui couvre une échelle d'au moins 1 à 100. En contraste avec cette large variation on note : 1) la constance générale du rapport entre les deux cultures à 660 et 330 $\mu l l^{-1}$ de CO_2 qui évolue entre 1,4 et 1,6. C'est un résultat important de l'étude /5/ qui démontre la permanence dans le temps de l'effet de stimulation de croissance par le CO_2. 2) La précision relative possible est surprenante. Pendant des périodes de 24 jours consécutifs une comparaison de croissance peut être appréciée à mieux que 1% près (1,394 $\pm$ 0,005). Il faut considérer que cela est dû non seulement à une bonne symétrie dans les conditions expérimentales mais aussi à un parfait synchronisme du développement des deux lots de plantes. En effet une avance de croissance d'un ou deux jours aurait provoquée une variation importante du rapport. Par exemple l'écart serait de 25% par jour de déphasage dans la période des 30 premiers jours après semis. D'ailleurs la légère variation des jours 25 est attribuée à l'avance dans la croissance foliaire du lot élevé en CO_2 enrichi cet effet est résorbé lorsque la surface totale devient grande (indice foliaire > 2), l'assimilation du couvert fermé devenant de plus en plus indépendante de la surface. Il ne reste que l'effet du CO_2 sur la Photosynthèse à surface donnée. III) Le temps d'établissement du couvert, très variable selon le type de culture, pourrait jouer de façon décisive pour amplifier l'effet du CO_2 en ajoutant l'effet d'accroissement de la surface foliaire à celui de stimulation de la Photosynthèse. Dans notre cas le temps d'établissement du couvert fermé est de près de 40 jours soit environ le double de celui de la culture à haute densité. Cela n'a pas modifié le résultat final. IV) Une autre observation d'ordre plus théorique concerne un critère d'analyse de croissance très utilisé /16/ le taux de croissance relative (Relative growth Rate ou RGR) $RGR = dw/wdt$ (1) où w est le poids sec produit et t le temps. On peut identifier le cumul de CO_2 assimilé à la biomasse - ce qui est justifié surtout si on considère la bonne corrélation entre consommation d'éléments minéraux majeurs et Photosynthèse étudiée plus loin -. Dans le domaine illustré par la figure 5 les RGR calculés pour les deux cultures sont pratiquement identiques alors que l'on constate une différence de croissance de près de 50%.

Cependant les deux lots étant identiques au départ, au stade graine, il faut qu'à un certain moment apparaisse une différence qui doit modifier le RGR. Effectivement une analyse plus fine et plus précoce /5/ montre que c'est dans la première décade que le RGR est légèrement différent et crée la différence conservée par la suite avec une égalisation du RGR. Cette égalisation est évidemment la conséquence de l'affinité entre les deux courbes.

En effet quel que soit $w_1 = f(t)$, si $w_2 = k.f(t)$ alors $RGR_1 = dw_1/w_1dt = f'(t)/f(t)$; $RGR_2 = kf'(t)/kf(t) = RGR_1$ ce qui démontre les limites voire le peu de pertinence du RGR dans les comparaisons de croissance. V) Certains auteurs /1/ (p.89) ont émis l'hypothèse, basée sur des théories de croissances végétales , que l'effet du CO_2 pourrait engendrer de grande différence de production de biomasse par amplification "exponentielle" au cours du temps de la différence de photosynthèse. Ce serait effectivement le cas si la RGR était continuellement plus élevée chez le lot enrichi en CO_2. On constate que ce n'est pas le cas chez le blé dans nos conditions de forte ou faible densité. Ces résultats contredisent également l'hypothèse inverse, tirée d'un affaiblissement de la stimulation due à l'effet du CO_2 au cours du temps /17/.

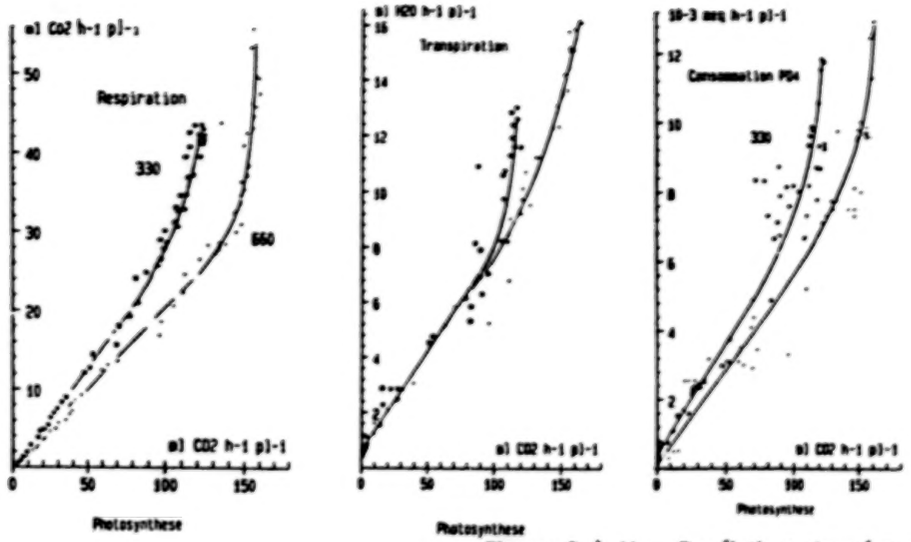
III - Relations entre les activités physiologiques

a) En phase quasi-stationnaire ou homéostatique. Les relations observées pendant la croissance entre les différentes activités physiologiques mesurables Respiration, Transpiration, Nutrition et la Photosynthèse sont portées figures 7 à 11. Pour chacune d'elles on note une phase de proportionnalité stricte avec la photosynthèse et une période pour laquelle ces activités augmentent plus vite que la photosynthèse. C'est particulièrement le cas de la respiration et dans une moindre mesure de la transpiration.

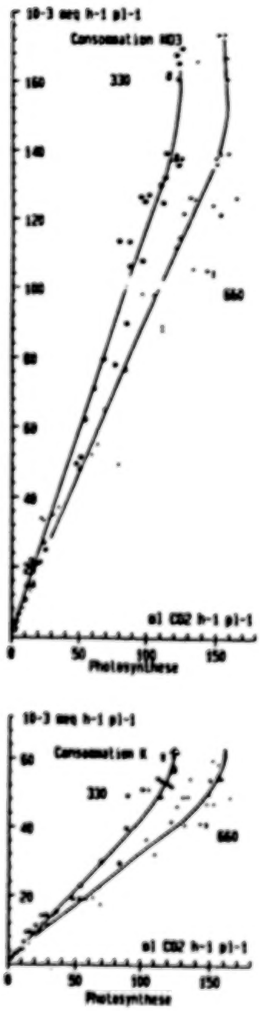
L'explication est à rechercher dans le fait que la photosynthèse est limitée, à indice foliaire élevé, par la surface éclairée proche de la surface au sol de la culture. La Transpiration et surtout la Respiration continuent à augmenter avec la surface réelle des feuilles. Dans le cas de la respiration il serait possible comme pour le Maïs /18/ de faire la part d'une respiration de croissance proportionnelle à la photosynthèse et la part d'une respiration de maintenance qui augmente avec la biomasse accumulée. Les coefficients de corrélation sont très voisins de ceux observés sur une culture à haute densité / 6/*.La vitesse de respiration nocturne est 25% de la vitesse de photosynthèse et les consommations de NO_3 , PO_4 , K , sont respectivement de 27, 1.7, 9.9, mmol/mole de CO_2. La transpiration est dans nos conditions de 100 mole/mmole CO_2.

b) Effet de la concentration en CO_2. L'effet du CO_2 n'est pas le même sur la photosynthèse et sur les autres activités mesurées ici. Pour une même photosynthèse on note une réduction de 20% de la respiration et de 19% de la transpiration ce qui peut être un avantage. Par contre la nutrition est différente, de l'ordre de 20 à 30% plus faible à bilan de CO_2 égal. Effet signalé dans les cultures en CO_2 enrichi /1/ qui peut être préoccupant pour les cultures en conditions spatiales réalisées a priori sous CO_2 très enrichi pour augmenter le rendement. L'essentiel est de savoir si cet effet est de nature à compromettre la quantité ou la qualité des produits commestibles.

*Signalons une erreur dans les figures V-4 et 5, /5/ et la figure 8, /6/ où il faut lire les valeurs de photosynthèse en ml $CO_2 h^{-1}$ plante^{-1} et non en mg $CO_2 h^{-1}$ plante^{-1}.



Figures 7 à 11 - Corrélations observées entre activités de respiration, de transpiration, de consommation d'éléments nutritifs et assimilation nette de CO_2 au cours de la croissance végétative du blé.



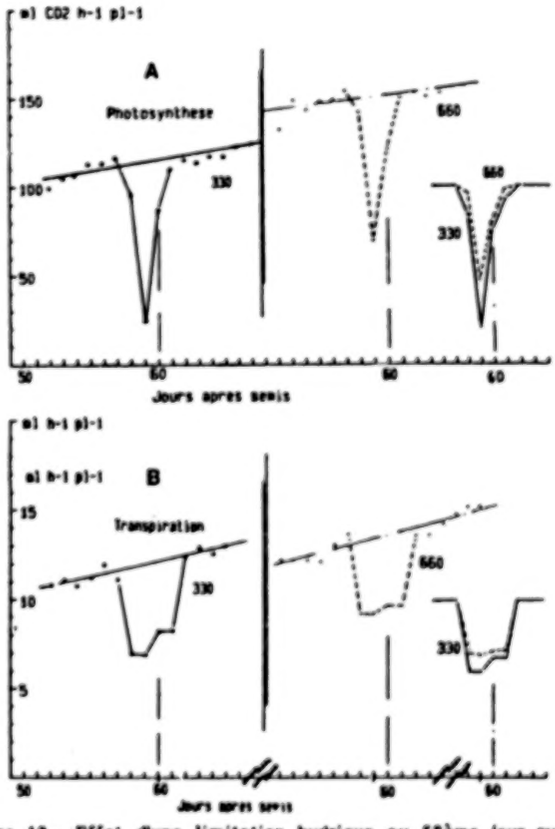


Figure 12 - Effet d'une limitation hydrique au 58ème jour sur la Photosynthèse (A) et de la Transpiration (B), pour les deux cultures à 330 et 660 µl l^{-1}. On a représenté, à droite, les variations normalisées pour faciliter les comparaisons.

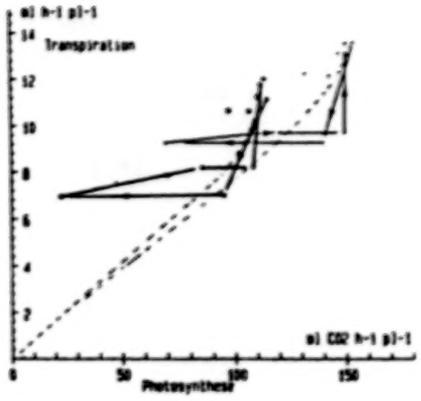


Figure 13 -

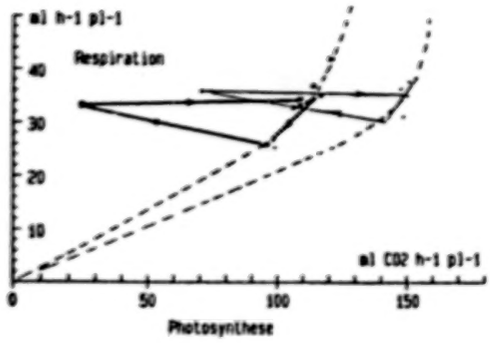


Figure 14 -

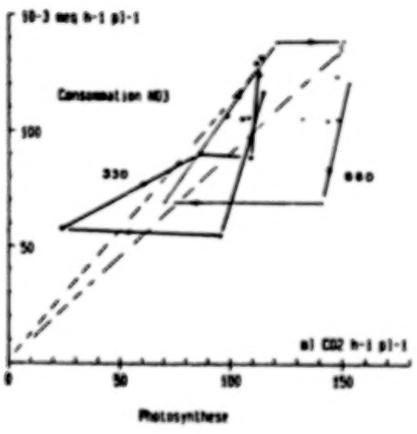


Figure 15 -

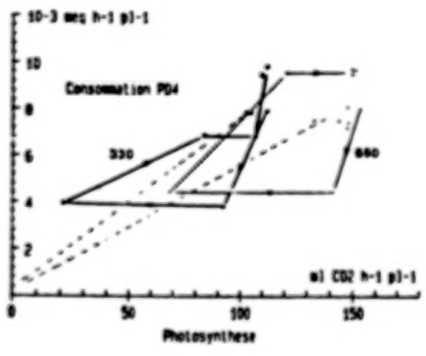


Figure 16 -

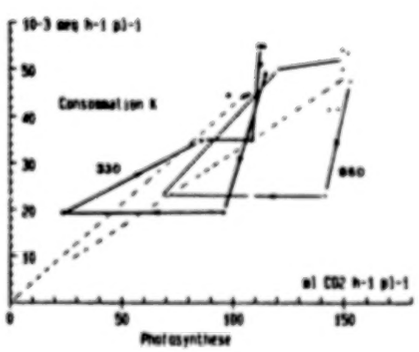


Figure 17 -

Figures 13 à 17 - Effet de la limitation hydrique du jour 58 sur les activités physiologiques. La représentation est celle des figures 7 à 11. Les corrélations mesurées en phase stationnaire sont rappelées en pointillé.

c) Relations observées en régime perturbé. On se propose d'examiner le cas de la limitation hydrique du jour 59 pour lequel l'apport journalier de solution nutritive a été accidentellement réduit au niveau de la transpiration journalière normale. Comme la transpiration intervient majoritairement pendant la phase éclairée (14 h) cela a conduit à une limitation effective de 14/24 pendant cette période soit une contrainte d'environ 60% de la transpiration. L'apport nocturne a percolé et s'est maintenu à 25% de la transpiration initiale en permettant de poursuivre la mesure de la consommation minérale.

Photosynthèse et Transpiration. Les figures 12a et 12b décrivent les variations des P et T qui ne sont comparables ni en amplitude ni en phase ou en durée. Pour les deux cultures la réduction de transpiration est plus faible mais plus longue que celle de la photosynthèse.

Ceci est en contradiction avec les résultats de Wong et col. /3, 4/ qui observent dans de nombreux cas et en particulier lors de stress hydriques une relation linéaire entre la photosynthèse et la conductance stomatique c'est à dire, en condition constante, entre Photosynthèse et Transpiration. On a choisi de représenter fig. 13 un type de relation non linéaire observée en régime perturbé. Dans notre cas la transpiration est d'abord réduite sans modification importante de photosynthèse qui est alors profondément diminuée à transpiration constante. La restauration suit un chemin inverse. Au bilan on note une perte de photosynthèse cumulée de 115% d'une photosynthèse journalière normale et de 145% de la transpiration journalière, soit qui est en faveur d'une légère amélioration de l'efficacité de l'eau.

Respiration. La figure 14 montre la réponse de la respiration qui est également éloignée de la fonction observée en régime homéostatique. Son activité est beaucoup moins perturbée que la photosynthèse ce qui est traduit par un parcours situé au dessus de cette fonction. On note un retour à la normale par valeur supérieure indiquant probablement une activité de restauration.

Nutrition. Les différentes réponses des consommations minérales sont illustrées fig.15, 16, 17. Elles présentent des caractéristiques très voisines entre elles. Leur signification est renforcée par la similitude d'effet sur l'expérience à CO_2 élevé. On peut distinguer 1) une baisse d'activité plus rapide que celle de la photosynthèse avec un retour également plus rapide. Cet effet est traduit par un parcours en boucle. Le sens de rotation du parcours indique une avance de phase de la variation de nutrition par rapport à la photosynthèse. Il n'y a pas au bilan une très nette variation du rapport nutrition/photosynthèse ce qui est manifesté par un parcours situé de part et d'autre de la fonction du régime stationnaire.

Une analyse similaire de la perturbation provoquée par une réduction de lumière chez le Maïs /19/ montrerait une réponse en retard de phase des variations de nutrition par rapport à la photosynthèse. On ne peut donc pas généraliser ce type de comportement qui n'est qu'un exemple de régulations complexes qui interviennent entre parties aériennes et racines. Il faut simplement retenir ici que les variations de nutrition minérale ne suivent strictement ni la photosynthèse ni la transpiration en régime variable ni la loi établie en régime quasi stationnaire.

CONCLUSION

Ces expériences apportent trois types de contributions. I) Dans la recherche en instrumentation et en méthode, une illustration est donnée des possibilités de mesure quantitative automatisée sur matériel vivant avec des précisions dans l'analyse de croissance qui sont comparables aux mesures physiques sur la matière inanimée. II) Sur le plan physiologique une démonstration est faite de la possibilité de forte différence de vitesse de croissance sans différence appréciable de vitesse de croissance relative. C'est précisément le cas de l'effet de l'augmentation de concentration en CO_2 qui est constant au cours du temps dans la phase végétative étudiée ici. Les deux autres types d'effet avancés par certains auteurs d'un effet "exponentiel" s'accroissant avec le temps ou à l'inverse d'un effet s'atténuant par "adaptation" au CO_2 sont exclus dans nos conditions pour le blé.

Dans l'analyse des relations entre les grandes activités physiologiques on montre qu'en régime homéostatique de croissance les relations suivent des lois simples pratiquement linéaires.

L'effet du CO_2 se traduit par une augmentation du rapport assimilation de carbone sur nutrition minérale qui peut avoir un effet important sur la qualité des produits commestibles formés.

En régime non stationnaire, dans le cas d'une limitation hydrique transitoire les variations des activités physiologiques s'écartent radicalement des fonctions précédentes, chacune (Respiration, Transpiration, Nutrition) ayant sa réponse propre.

a) Photosynthèse et Transpiration ne varient pas ensemble ce qui met en question la généralisation des résultats de Wong et col. 1985, sur les variations concomitantes entre conductance stomatique et photosynthèse. Le type de réponse se traduit par une amélioration transitoire de l'efficience de l'eau.

b) Les consommations de NO_3, PO_4 et K ne suivent ni la photosynthèse ni la transpiration. Leur variation par rapport à la photosynthèse est une fonction complexe que l'on peut traduire par une réponse d'amplitude voisine de la variation de photosynthèse mais en avance de phase par rapport à cette dernière.

c) Cette approche peut contribuer à prévoir les types de régulation à mettre en oeuvre dans un système de cultures en cycle écologique fermé étudié en vue des missions spatiales permanentes ou lointaines.

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N88-12269

UTILIZATION OF POTATOES IN BIOREGENERATIVE LIFE SUPPORT SYSTEMS

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ABSTRACT

Data on the tuberization, harvest index, and morphology of 2 cvs of white potato (Solanum tuberosum L.) grown at 12, 16, 20, 24 and 28°C, 250, 400 and 550 μmol s^{-1} m^{-2} photosynthetic photon flux (PPF), 350, 1000 and 1600 μl l^{-1} CO_2 will be presented. A productivity of 21.9 g m^{-2} day^{-1} of edible tubers from a solid stand of potatoes grown for 15 weeks with continuous irradiation at 400 μmol s^{-1} m^{-2}, 16°C and 1000 μl l^{-1} CO_2 has been obtained. This equates to an area of 34.3 m^2 being required to provide 2800 kcal of potatoes per day for a human diet. Separated plants receiving side lighting have produced 32.8 g m^{-2} day^{-1} which equates to an area of 23.6 m^2 to provide 2800 kcal. Studies with side lighting indicate that productivities in this range should be realized from potatoes. Glycoalkaloid levels in tubers of controlled-environment-grown plants are within the range of levels found in tubers of field grown plants. The use and limitation of recirculating solution cultures for potato growth is discussed.

BACKGROUND

The white potato, Solanum tuberosum L., is one of eight plant species identified in the U.S. for primary consideration as useful crops /1/ for a biogenerative or controlled ecological life support system (CELSS). The others are wheat, rice, peanut, soybean, sweet potato, sugar beet and lettuce. Potatoes are unique among these crop species in that the edible portions of the plant develop as tubers below the soil surface on underground, horizontally-growing stems. The potato was selected for inclusion in a space life support system because of several desirable characteristics.

Productivity: Foremost among these characteristics of the potato is its very high productivity of digestible food per unit area per unit time, a level that is as high as any other species in this selected group except sugar beets (Table 1). The calculations shown in Table 1 were based on maximum harvested yield data for different countries of the world /2/ utilizing a typical field growing period and adjusting for the water content, for the portion of the produce that is not eaten, and for the portion that is not digestible /3/.

Harvest index: Of significant importance in a CELSS is the fact that potatoes have a very high harvest index, 80% (Table 1). This means that 80% of the dry matter accumulated by a mature potato plant is allocated to tubers and only 20% to the inedible stems, leaves and roots. Thus, the expenditure of energy required for recycling the inedible portions of a potato plant will be significantly less than for species that have a lower harvest index. Typically the harvest index of species for which vegetative tissues are consumed is high (eg. potato, lettuce, sweet potato), whereas the harvest index of species for which reproductive tissues, as seeds and fruits (e.g. wheat, rice, peanuts), are consumed is usually significantly less. It is important to note, however, that the harvest index does not necessarily represent the total usable portion for most crops, as a portion of the harvested product is often discarded in the form of husks or peeling; this unusable portion can be as high as 25% for rice and peanuts. Also, the harvested portion that is consumed includes a fraction of non-digestible dry matter. This is highest in sugar beets and lettuce.

The edible portion of potatoes, the tubers, consists mostly of the carbohydrate, starch. However about 9% of the dry weight of tubers is protein. These proteins have a reasonable balance of all required amino acids and are high in lysine compared to most of the other selected crops. In fact, the quantity of protein present in potatoes is sufficient to satisfy the total protein requirement of a person if all the dietary energy requirements were met with potatoes.

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TABLE 1 Productivity of various food crops proposed for utilization in CELSS.

Crops		Field Production ($g\ m^{-2}$)	Growing Period (days)	Edible or Usable Portion (%)	Water Content (%)	Digestible Food	
English Name	Latin Name					Edible Portion (%)	Dry Weight ($g\ m^{-2}\ day^{-1}$)
Wheat	Triticum sativum	738	100	95	14.0	98.0	5.91
Brown Rice	Oryza sativa	789	110	75	14.4	89.5	4.12
Sugar Beets	Beta vulgaris	6440	160	100	75.0	76.8	7.26
Peanuts	Arachis hyponaea	360	150	75	5.0	93.8	1.61
Soybeans	Glycine max	283	140	95	10.0	94.5	1.64
White Potatoes	Solanum tuberosum	5492	150	90	79.8	85.9	5.72
Sweet Potatoes	Ipomea batatas	2186	120	90	70.6	86.8	3.92
Crisphead Lettuce	Lactuca sativa	3474	60	90	95.1	81.1	2.32

Culinary: Potatoes, along with all of the selected crops, are palatable and acceptable to most people. This contrasts to certain other food species that are highly productive but which are not as palatable for a significant proportion of humans.

Preparation: Potatoes require a minimum of processing to make them useful as foods thus making it possible to minimize the "kitchen" facility and energy requirements in a CELSS unit. The tubers are ready for kitchen use when harvested from the plants. They also can be prepared in many different ways so that they can be utilized in nearly every meal. For example, potatoes can be eaten raw, boiled, baked, fried, microwaved, french fried, scalloped or processed into chips, sticks and puffs. In addition, a flour can be made from potatoes and used in many ways. Potatoes can be stored for periods up to 6 months as fresh tubers or for longer periods as a frozen or dried product.

Information: There is extensive knowledge on the cultural requirements and physiology of potatoes with entire books dedicated to this in many different countries, and there are collections of diverse germ plasm available from the plant introduction stations in many countries from which diverse genetic traits can be obtained to breed potatoes best suited for controlled environment production. Furthermore, potatoes can be easily maintained from stem cuttings in sterile tissue culture, and less than 0.2 m^2 of space would be required to maintain planting stock and transplants to grow the food (energy and protein) requirements for one person. Thus disease-free uniform plant stock can be easily propagated and maintained for an operational CELSS.

RESEARCH

Production: Potatoes have been grown successfully in controlled environments under electrical lighting, without any sunlight, by researchers on numerous occasions. We have utilized cool white fluorescent lamps exclusively to provide normal growth and development of potato plants. The following graph (Figure 1) depicts the production obtained from potato plants of the Norland cultivar in a controlled environment room under continuous irradiation at a PPF (photosynthetic photon flux) of 400 $\mu mol\ s^{-1}\ m^{-2}$ (400 $\mu E\ s^{-1}\ m^{-2}$) and at 16°C temperature. Individual plants were maintained in 38-liter containers filled with a potting mixture and watered four times each day with nutrient solution /4/. Each plant was constrained by a wire netting that provided 0.2 square meters of cross-sectional area. Successive harvests demonstrated that tuber production continued to increase until the last harvest at 147 days but that the productivity in grams $m^{-2}\ day^{-1}$, as shown by the values in parenthesis, peaked at 126 days (32.46 $g\ m^{-2}\ day^{-1}$) although the productivity was essentially level from 105 days to 147 days. In an operational CELSS, there would likely be an advantage to maintaining plants for as long a growing period as possible to reduce the frequency of planting and harvesting operations.

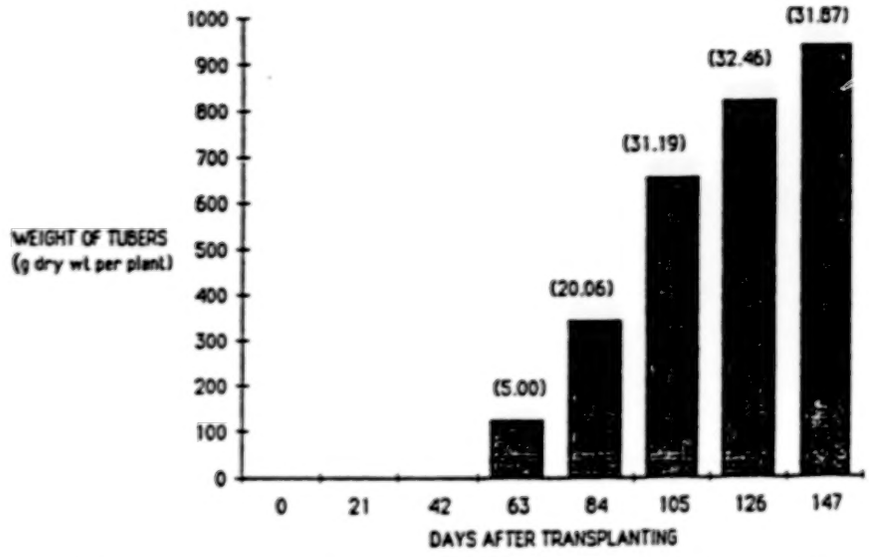

Fig. 1. Tuber production of Norland potato plants grown at 16°C and 400 μmol s^{-1} m^{-2} PPF. Plants were individually confined within a 0.2 m^2 fenced area. Values in parentheses are g dry matter m^{-2} day^{-1}.

Light interception: Under normal growth, potatoes very quickly begin branching and spread out to form a solid stand. When plants are spaced on 50-cm centers, there is total light interception by 6 weeks after planting. After this time, there is essentially no light penetration through a solid canopy as shown in Figure 2 and thus irradiation is maximally utilized from 6 weeks on. Because the leaves of potato tend to be nearly horizontal, they are capable of intercepting light very effectively. Figure 3 shows that when potato plants were grown in a solid stand under a 12-hr photoperiod, a leaf area index of two (i.e. two layers of leaves above a unit area of ground) effectively intercepted over 90% of the incident radiation. This characteristic permits potatoes to grow well and maintain high productivity at relatively low irradiance levels.

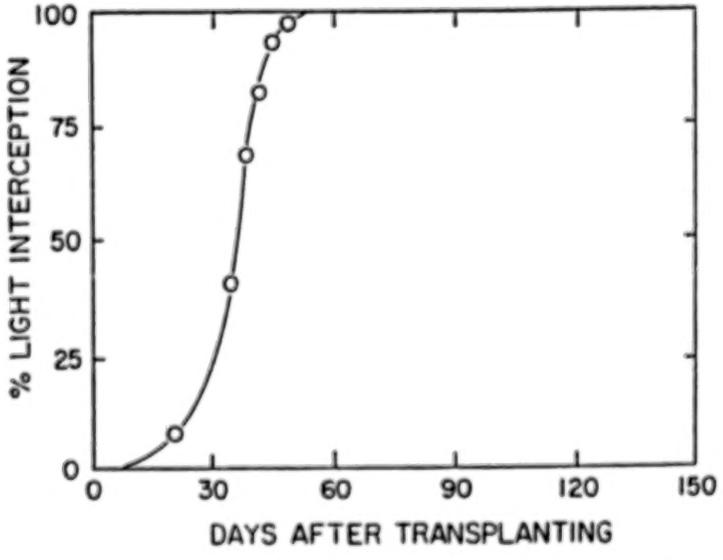

Fig. 2. Light interception by potato plants as measured at soil surface midway between pots spaced 0.5 m apart.

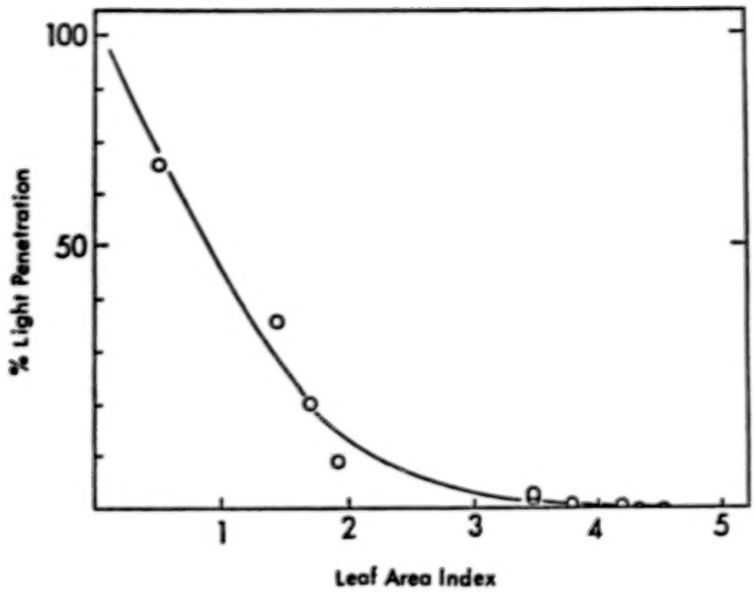

Fig. 3. Penetration of light through a canopy of potato leaves as a function of leaf area index (i.e. area of leaves above a unit area of ground). Potato plants were grown under a 12-hr photoperiod using cool white fluorescent lighting.

Lighting: It has long been accepted and demonstrated that short photoperiods, e.g. less than 12 hours, encourage tuberization of potatoes, however we have found that continuous irradiation (24 hr) will provide tuberization if sufficient light intensity is provided. A photosynthetic photon flux level (PPF) of 400 μmol s^{-1} m^{-2} maintained continuously, provided 60% more tubers than with the same level maintained for a 12-hr period and 12-hr dark each day (Table 2). There was evidence at the 6-wk harvest that tuber development began slightly sooner with 12 hr irradiation but in all succeeding harvests, tuber production was significantly greater under continuous irradiation than under 12 hr irradiation. Even the harvest index of plants grown under continuous irradiation was high and essentially the same as under 12-hr irradiation (Table 2). Note that the tuber production under continuous irradiation is only about 50% greater than under 12 hr irradiation, yet the total photosynthetically active radiation received each day with continuous lighting is double the amount obtained under the 12-hr photoperiod. This suggests that there is greater irradiant energy conversion efficiency with the 12-hr photoperiod, or in other words, there is a greater dry weight increase for a given quantity of irradiation.

TABLE 2 Dry Weight and Harvest Index of Norland Potato Plants Grown Under Different Irradiance Durations.

Light (hrs)	Dark	Dry Weight Tubers	Leaves	Stems	Harvest Index (%)
		(g per plant)			
12	12	617	134	9	80
24	0	937	167	37	81

16°C at PPF level 400 μmol $s^{-1}m^{-2}$ for 21 weeks

Evidence that long photoperiods do suppress tuberization was seen in an experiment comparing 400 μmol s^{-1} m^{-2} PPF for 12-hr with 200 μmol s^{-1} m^{-2} for 24 hr in which the same total amount of irradiation was provided over a 24-hr period. There was essentially no tuberization under the continuous light treatment and good tuberization with a 12-hr light treatment (Table 3). However, this long photoperiod suppression of tuberization can be overcome by increasing the irradiance level from 200 to 400 μmol s^{-1} m^{-2}, as shown in the middle treatment of this table. It is noteworthy that when total plant growth was compared between 400 μmol s^{-1} m^{-2} for 12 hrs and 200 μmol s^{-1} m^{-2} for 24 hrs for these 6-week old plants, there was less total dry weight and poorer energy conversion efficiency with the 12-hr treatment (Table 3).

TABLE 3 Dry Weight of Norland Potato Plants Grown at Different Irradiance Durations and Levels.

Irradiance		Dry weight per plant	
Duration (hrs)	Level of PPF ($\mu mol\ s^{-1}m^{-2}$)	Total biomass (g)	Tubers (g)
12	400	53	12.2
24	400	125	19.6
24	200	80	0.4

20°C for 6 weeks

A significant concern in the use of continuous irradiation is that only about one-half of the cultivars we have tested will develop and tuberize normally. This includes the cultivars Norland, Russet Burbank, Denali, Atlantic, and Snowchip. Other cultivars have become chlorotic after two weeks of growth, producing small leaves and greatly stunted growth. This injury response has been seen with Kennebec and Superior cultivars. A partial chlorosis and stunting also has occurred with Norchip cultivar.

Our research to date has not established the most useful level of irradiation for potatoes. Limited studies with continuous irradiation at 550 $\mu mol\ s^{-1}\ m^{-2}$ have provided little tuber or total dry matter gains over 400 $\mu mol\ s^{-1}\ m^{-2}$, thus we are hypothesizing that 400 $\mu mol\ s^{-1}\ m^{-2}$ when given continuously, is close to a maximum useful level. However, we suspect that higher irradiance levels may be beneficial if irradiation is provided for shorter photoperiods.

Temperature: Tuberization is very dependent on the temperature under which the plants are grown. Tuberization is stimulated by cool temperatures and shown to be maximum between 16 and 20°C (Table 4). At 28°C no tubers formed on the plants. The total dry weight followed a similar pattern as tuberization. The stem length of plants was significantly shorter with decreasing temperatures and consequently, the harvest index was highest and most desirable at the coolest temperatures.

TABLE 4 Dry Weight, Stem Length, and Harvest Index of Norland Potatoes Grown Under Different Temperature Levels.

Temperature	Dry Weight		Stem Length	Harvest Index
	Tubers	Total Plant		
(°C)	(g per plant)		(cm)	(%)
12	73	123	18	59
16	123	209	37	59
20	96	186	48	51
24	2	151	74	1
28	0	116	94	-

Continuous PPF of 400 $\mu mol\ s^{-1}m^{-2}$ for 8 weeks.

Carbon dioxide: Elevation of carbon dioxide concentrations has been found to be only of marginal benefit to potato plants when grown under continuous irradiation at 400 $\mu mol\ s^{-1}\ m^{-2}$. There has been little or no gain in tuber dry weight either in short-term studies of 8 weeks (Table 5) or long-term studies of 15 to 18 weeks. We have obtained some evidence that the late-maturing cultivar, Russet Burbank, derived some benefit from CO_2 increases but no evidence that the early-maturing cultivar, Norland, benefits from CO_2 increases. The lack of enhanced growth from CO_2 enrichment suggests that we have approached the physiological limit of the growth capacity of the potato plant under the continuous irradiance levels of 400 $\mu mol\ s^{-1}\ m^{-2}$.

TABLE 5 Tuber Dry Weight of Potato Plants Grown Under Different Carbon Dioxide Concentrations.

Concentration	Tuber Dry Weight: Norland cv	Russet Burbank cv
($\mu l\ l^{-1}$)	(grams per plant)	
400	85	88
1000	74	103
1600	81	105

20°C at PPF level of 400 $\mu mol\ s^{-1}m^{-2}$ for 8 weeks

Productivity: The tuber production from our research studies can be utilized to calculate the area and lamp power required to provide the average energy requirements, 2800 kcal per day, for one person living in a space colony. Knowing that one gram of dry weight of potatoes contains 3.73 kcal of energy, then 750 grams of dry weight of potato would be required for one person per day. We have obtained a production of 21.9 $g\ m^{-2}\ day^{-1}$ from a solid stand of potatoes. Thus the area required for one person to fulfill energy and protein requirements is 34.3 m^2. If the production from the single plants in separate cages (32.8 $g\ m^{-2}\ day^{-1}$) could be obtained from a solid stand, then only 23.6 m^2 would be needed to meet the requirements for one person. We feel that this latter figure is a realistic estimation of space requirements needed, and only requires that lighting systems be designed to provide irradiation to all sides of the plant.

In a commercial plant growing facility in the United States, a light level of 400 $\mu mol\ s^{-1}\ m^{-2}$ requires 304 lamp watts m^{-2} using high pressure sodium lamps. Using this efficient conversion, (23.6 m^2 x 304 wm^2) approximately 7.2 kw of electricity would be required per person.

Stem length: Potato growth in a life support system will likely be constrained by the total volume of the growing modules to be utilized. A concern in use of potatoes for space systems is that potatoes have a tendency to grow long stems. Stems can be in excess of a meter in length on certain cultivars and this extension is encouraged by elevated temperatures, long photoperiods, and low light levels. It has been found that early-maturing cultivars tend to have shorter stems than late-maturing cultivars apparently because the early-maturing cultivars stop producing shoot growth when tuberization is vigorous wheras late-maturing cultivars continue active shoot growth during tuberizataion. We have seen that caged plants receiving side lighting exhibit less stem elongation. This has encouraged us to develop a lighting system to provide supplemental irradiation just above the soil level on each side of the plants using 1500 mA CWF lamps. Plants with side lighting developed short main stems and branches yet produced the same total dry weight and similar tuber dry weight as plants grown without side lighting. We are pursuing further studies using within canopy lighting to determine whether this procedure will not only reduce stem length, but also irradiate the plants more efficiently and reduce the space required for fulfilling production needs.

Recirculating nutrient: Significant effort has been directed toward developing a means of growing potatoes in recirculating nutrient solutions. These systems are being studied to provide effective nutrient recycling and to examine the possibility of harvesting individual tubers as each matures and thus hopefully maintaining plants in a continuously productive state. Potato plants grow well in many different types of recirculating systems but in most systems we have studied, plants failed to tuberize normally, exhibiting delayed tuber initiation and sometimes producing many but only small tubers.

The most successful system that we have utilized involves filling a 90 cm long X 60 cm wide and 20 cm high polyethelene tray with either calcined clay particles (arcillite) or sphagnum moss and slanting the tray so that nutrient solution (300 ml min^{-1}) can be added along one end of the tray and recirculated through holes on the opposite end of the tray (Figure 4). This approach produced rapid and good tuberization and studies are currently underway to determine the minimum depth of calcined clay required for effective tuberization in these trays. If a depth of less than 3 cm is effective, the potential for studying continuous tuber harvesting would be available.

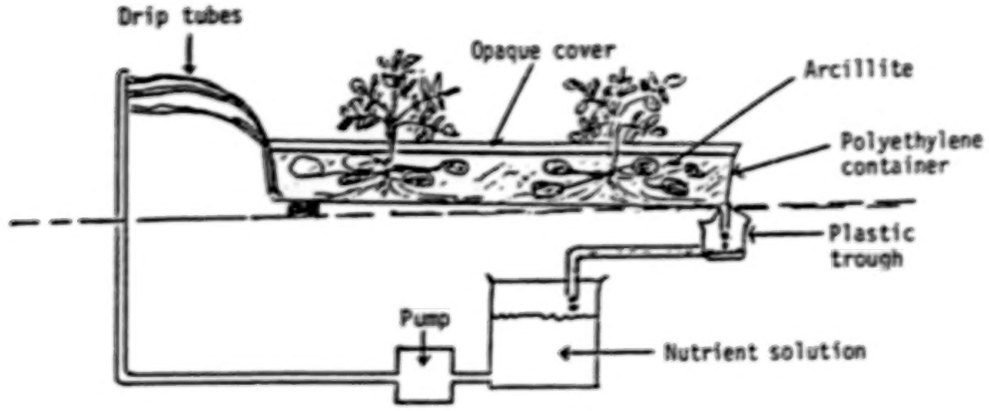

Fig. 4. Diagram of recirculating tray culture system for potato production. Tray filled with particles of calcined clay (arcillite) and fed continously at one end with 300 ml min^{-1} of nutrient solution.

In other studies when solution was recirculated through containers filled with nutrient solution and the plant roots and lower stem immersed, tuber initiation and development was greatly slowed. When the solution level was lowered to expose the lower stem area, tuberization was still slowed. However, when the solution level was lowered and the area around the lower stem filled with sphagnum moss, tuberization was essentially normal.

To date, mist culture systems ("aeroponics") also have not provided effective tuberization. A mist system was developed with a spinning impellor located at the level of the tuber forming stems, which continuously atomized solution onto the underground stems and the root systems (Figure 5). The solution was collected in the base of the misting compartment for recirculation. Although tuberization was generally slowed it was possible to stimulate tuber development if the system was stopped for a sufficient period to slightly wilt the plants or by complete removal of nitrogen from the recirculating solution.

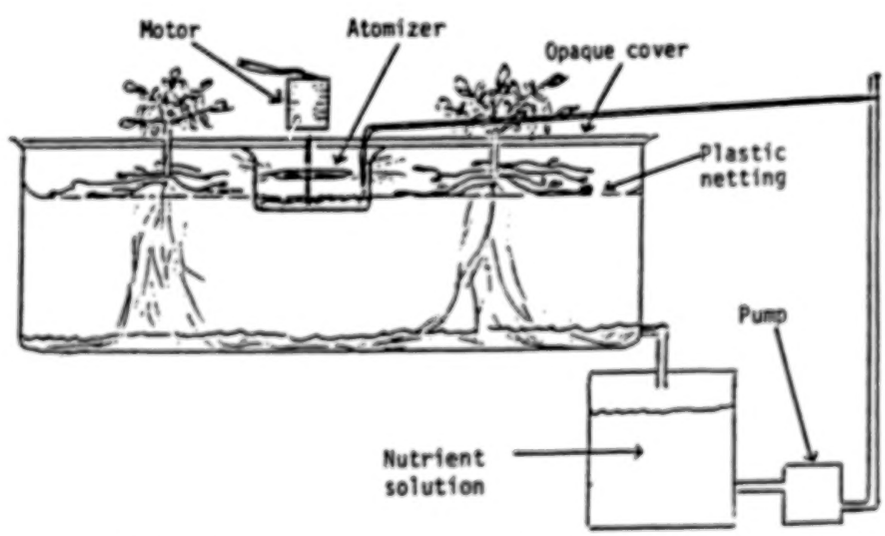

Fig. 5. Diagram of mist culture system developed for potatoes utilizing a spinning atomizer with recirculating nutrient solution.

Glycoalkaloid levels: A significant concern in our research effort has been the possibility that the unique growing procedures utilized in maximizing production for CELSS would increase the toxic glycoalkaloid level in the tubers. The glycoalkaloids, chaconine and solanine, are prevalent in potatoes and are known to vary in concentrations depending upon the conditions during growth /5/. Thus sample tubers from all experiments are regularly analyzed for these glycoalkaloids and we are gratified to report that we have found no levels under any growing conditions studied that exceed the average levels found in field potatoes. In our studies the levels found have been 0.03 to 0.07 mg per gram fresh weight for chaconine and 0.02 to 0.05 mg per gram fresh weight for solanine.

Summary: The research to date with potatoes suggests that this crop has a significant place in an operational CELSS and can be utilized either alone or in combination with other food crops, to fulfill a significant portion of the energy and protein requirements of humans in space. The high potential productivity of nutritious tubers, easy propagation, and the high harvest index of the potato plant make it a particularly strong candidate. Thus potatoes, along with other useful crops, can serve a vital role in a life support system ... to feed astronauts, provide needed oxygen, and remove carbon dioxide

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N88-12270

WHEAT PRODUCTION IN CONTROLLED ENVIRONMENTS

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ABSTRACT

Our goal is to optimize conditions for maximum yield and quality of wheat to be used in a controlled-environment, life-support system (CELSS) in a Lunar or Martian base or perhaps in a space craft. With yields of 23 to 57 g m^{-2} d^{-1} of edible biomass, a minimum size for a CELSS would be between 12 and 30 m^2 per person, utilizing about 600 W m^{-2} of electrical energy for artificial light. Temperature, irradiance, photoperiod, carbon-dioxide levels, humidity, and wind velocity are controlled in state-of-the-art growth chambers. Nutrient solutions (adjusted for wheat) are supplied to the roots via a recirculating system that controls pH by adding HNO_3 and controlling the NO_3/NH_4 ratio in solution. A rock-wool plant support allows direct seeding and densities up to 10,000 plants per $meter^2$. Densities up to 2000 plants m^{-2} appear to increase seed yield. Biomass production increases almost linearily with increasing irradiance from 400 to 1700 µmol m^{-2} s^{-1} of photosynthetic photon flux (PPF), but the efficiency of light utilization decreases over this range. Photoperiod and temperature both have a profound influence on floral initiation, spikelet formation, stem elongation, and fertilization. High temperatures (25 to 27°C) and long days shorten the life cycle and promote rapid growth, but cooler temperatures (20°C) and shorter days greatly increase seed number per head and thus yield (g m^{-2}). The life cycle is lengthened in these conditions but yield per day (g m^{-2} d^{-1}) is still increased. We have evaluated about 600 cultivars from around the world and have developed several breeding lines for our controlled conditions. Some of our ultra-dwarf lines (30 to 50 cm tall) look especially promising with high yields and high harvest indices (percent edible biomass).

INTRODUCTION

What are the size and energy constraints on a bioregenerative system that utilizes photosynthesis of higher plants to capture light energy and convert it to the chemical bond energy of food needed to support a human being? Imagine that the human being, thanks to advanced gene-transfer techniques, could personally manage the photosynthetic conversion, absorbing and converting 100% of visible light energy into a form that could be utilized by the body. Assume that the human energy requirement is 11,700 kJ d^{-1} (2800 kcal d^{-1}). The solar constant above the earth's atmosphere is 1.36 kW m^{-2}. About half of that is photosynthetically active radiation (PAR): 0.68 kW m^{-2} or 0.68 kJ m^{-2} s^{-1}. The 11,700 kJ d^{-1} required by our hypothetical astronaut is equal to 0.135 kJ s^{-1} (kW), which divided by the 0.68 kJ m^{-2} s^{-1} PAR gives about 0.2 m^2 $person^{-1}$.

A human being intercepts an area of about 0.5 to 0.9 m^2 of sunlight, if the rays are from the front or back and normal to the long axis of the body. Thus, our hypothetical space traveler could do fairly well if he or she wore few clothes and stayed in the sun all the time in a position that would intercept about half the incoming radiation.

Not even the most efficient plant can convert 100% of absorbed PAR into the chemical-bond energy of food, however. Indeed, the theoretical maximum efficiency for photosynthesis is about 25% (based on 8 photons per molecule of fixed CO_2), and 13.5% (15 photons per molecule CO_2) is a more realistic figure. Thus, our photosynthesizing astronaut--or the best imaginable canopy of photosynthesizing plants under the best of conditions--would require about 0.8 m^2 (25% efficiency) or 1.5 m^2 (13.5% efficiency) to produce the 11,700 kJ d^{-1} that

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*Author presenting paper. Research reported in this paper was supported by National Aeronautics and Space Administration Grant NCC-2-139, administered through the Ames Research Center, Moffet Field, California. Support was also received from the Utah Agricultural Experiment Station. This is Utah Agricultural Experiment Station Paper 3324.

are required. So the photosynthesizing space dweller is already in trouble. Animals lack sufficient surface to be directly supported by photosynthesis.

Under full sunlight, few plants photosynthesize at anything approaching 13.5% efficiency. If we reduce the irradiance to half of full sunlight, we will need at least 3.0 m^2, and if our plants are in the light only about half the time (12 h d^{-1}), the value becomes 6.0 m^2. And if only about half of the biomass produced by the plant can be eaten as food (50% harvest index), the value goes up again to about 12 m^2.

If the light for photosynthesis must be generated electrically, this would require about 600 W m^{-2} if high pressure sodium (HPS) lamps are used (37.6% efficient) in the most efficient reflectors (90% efficient). This would produce a photosynthetic photon flux (PPF) of 1000 micromoles of photons per square meter per second (μmol m^{-2} s^{-1}; 200 W m^{-2} PAR), which is about half of full sunlight at the earth's surface. To irradiate 12 m^2 would require 7.2 kW. In a functioning controlled, ecological, life-support system (CELSS), additional electric power would be required to operate the environmental control system, various aspects of food processing, repair and maintenance of the equipment, waste disposal, and mineral-nutrient regeneration for the plants. In calculating the total energy requirement, energy expended by the space farmers would also have to be considered. So far, our NASA-funded CELSS project has been concerned only with the food production part of a CELSS.

Consider another approach to the calculation: One-hundred grams of typical, whole-grain, hard-red spring wheat contain about 13 g of water, 14 g of protein, 2.2 g of fat, and 69.1 g of total carbohydrate (including 2.3 g of fiber). Bomb calorimeter studies indicate that the 100 g of wheat would provide 1647 kJ (394 kcal) of food energy, and if we assume that 94% of this energy is digestible, this would provide 1500 kJ (370 kcal). To provide the 11,700 kJ d^{-1} required by a human being, about 680 g d^{-1} of oven-dry wheat (780 g d^{-1} of typical wheat) or its equivalent in other food would be required. If this were to be produced in 12 m^2, yields would have to reach 57 g m^{-2} d^{-1}. If the production area was 30 m^2, then average daily production would need to be 23 g m^{-2} d^{-1}. Even if the 30 m^2 is doubled for safety, a moon farm about the size of an American football field (about 6000 m^2 including end zones) would support 100 inhabitants of Lunar City. Our objective has been to see what yields (g m^{-2} d^{-1}) can be achieved in a totally controlled environment and thus to test the reality of these figures.

In our studies /3/, we have taken a dual approach: agronomy/physiology and plant breeding. Which environmental and cultural factors are important to achieve high yields per unit of input energy and in the least possible area? How do these factors interact with each other and exactly how do they influence ultimate yield? Is it necessary to develop new cultivars for the special environments that can be produced in a CELSS? Can we breed cultivars for these new environments? The rest of this paper reports our efforts to answer these questions.

THE DEVELOPMENT OF HARDWARE

Much of our project has been concerned with the development of equipment and systems to achieve the necessary environmental control. These efforts fall logically into three categories: control of the foliar environment, control of the root environment, and the mechanics of supporting the plants.

The Foliar Environment

We began our studies with the purchase of three high-quality, controlled-environment growth chambers. These provide control of temperature, humidity, irradiance (PAR), photoperiod, and air flow. Control of air flow is achieved with variable speed fans and in all studies is regulated between 0.5 and 2 m s^{-1}. To increase irradiance levels so that they approach those of full sunlight, high pressure sodium lamps were added to all chambers. In one chamber, 5.2 kW of metal halide and HPS lamps were added to provide 2000 μmol m^{-2} s^{-1} PPF (full sunlight). In this chamber, a water filter below the lamps is used to reduce non-photosynthetic radiation. This water filter reduces total radiation by 37% without reducing PPF.

In addition to the three relatively small chambers (1 m^2 of growing space in each), we have built a six-subcompartment chamber specifically for photoperiod studies. The air conditioning and air delivery system is common to all six compartments, but they are separated from each other by barriers so that each compartment can experience its own photoperiod and irradiance level. The six lighting systems are exclusively HPS lamps, with the light filtered through 5 cm of chilled water. Temperatures in the 6 compartments remain within 0.2°C of each other.

We enrich carbon dioxide (CO_2) concentrations in all locations to 1000 μmol mol^{-1} of air. Our CO_2 enrichment system uses carbon dioxide from compressed gas cylinders and mixes it with air from above the building. This CO_2-enriched air is then piped to the chambers. An

infrared gas analyzer continuously monitors CO_2 levels in all chambers and greenhouse bays that are also part of this project (and which have been outfitted with CO_2 enrichment, HPS lamps to raise irradiance especially during winter, and a layer of flowing water over the top of the greenhouse glass). We do not use a feedback system to automatically control CO_2 levels, because we have found that CO_2 levels can easily be maintained at 1000 plus or minus 50 $\mu mol\ mol^{-1}$ with manual control.

One chamber has been sealed and outfitted with large cooling coils that circulate water below the air temperature in the chamber but above the dew point so that no liquid water condenses on the air conditioning system. In this chamber the root-zone environment is sealed to isolate it from the foliar environment. This means that photosynthetic rates of entire canopies of plants in the chamber can be measured (by monitoring input gas volumes and input and output CO_2 levels).

Root-zone Environment

A CELSS will ultimately need some kind of recirculating hydroponic system that maintains plant roots in an optimized environment. We have designed, built, and evaluated a hydroponic system that allows for nearly complete closure of the root-zone environment. The system contains four separate, root-zone compartments in each growth chamber, each with a surface area of 0.2 m^2 and a depth of 0.125 m. The volume of each compartment is therefore 0.025 m^3 (25 liters). The nutrient solution is pumped from a 0.1-m^3 reservoir. It flows through a distribution manifold in each of the root-zone compartments at a rate of 0.08 liter s^{-1}. The rapid flow rates help to provide both dissolved oxygen and good environmental uniformity between different root-zone compartments. The distribution manifold helps to ensure good uniformity within each compartment. The solution returns by gravity flow into a drain tube that is located in the center of the compartment, and then it drains back to the reservoir. Our measurements indicate that the solution is never less than 85% saturated with oxygen at all locations. The aeration is provided when the solution cascades back into the reservoir and causes considerable liquid/air contact by the bubbling action that occurs.

The total liquid volume of a system for a single growth chamber is 0.2 m^3. Each such system typically has a total dry biomass at harvest of ca. 3 kg. During the life cycle, each crop typically absorbs about 300 g of mineral elements from the solution and transpires 0.6 m^3 (600 liters) of water. The water and nutrients are replaced by adding a dilute refill solution that includes concentrations of the nutrients in proportion to their concentrations in the plant. All system components in contact with the solution are inert, including an epoxy-coated, magnetic-drive pump. The system is flushed between trials, cleaned with sodium hypochlorite, and then finally cleaned with acid.

We have now combined the three separate nutrient systems (one for each growth chamber) into a single system. Plants in all three chambers are thus supplied with the same nutrient solution. The liquid volume of the system could probably be reduced to minimize the mass, but our objective, thus far, has been to provide an optimum root-zone environment, rather than a small sized system.

The pH of the system is continuously monitored and controlled with an in-line pH electrode that opens a solenoid to introduce acid when the pH rises to 5.8 pH units. The pH change for young plants is very gradual, and nitric acid is added to maintain the pH between 5.5 and 5.8. As the ratio of plant mass to solution volume becomes larger, the pH changes more rapidly. After 25 days of growth we use a mixture of ammonium nitrate and nitric acid in the pH adjustment solution. The ammonium ion gradually decreases the pH as it is absorbed. As the ammonium is depleted from solution, the pH gradually rises again until it reaches pH 5.8, and the solenoid admits more control solution.

Plant Support

Much work on plant response to environment has involved individual plants, but virtually all crops used in a cells will be grown in dense canopies of plants to absorb as much light as possible. Plant response to environment is strongly influenced by the close presence of other plants, so it is imperative that CELSS research be conducted with canopies of plants. But how dense should the canopy be?

In several studies, we have found that increasing densities well beyond the optimum for field production (about 200 to 300 plants m^{-2}) has resulted not only in better light interception and increased biomass production but also in increased final yields of grain. We have therefore been concerned with methods of supporting the plants at high densities. We have now completed tests of a third-generation, germination and support system. Our first system was capable of supporting 550 plants per $meter^2$. We germinated the plants in vermiculite and transplanted them into foam plugs that were placed in a rigid styrofoam lid. This process required an hour to transplant 0.2 m^2 of plants. The second system held plants between bars and strips of foam. This allowed us to use plant densities of up to 1500

TABLE 1 The Effect of Photosynthetic Photon Flux (PPF) on Early Growth of Wheat

PPF $\mu mol\ m^{-2}\ s^{-1}$	PPF $mol\ m^{-2}\ d^{-1}$	Crop Growth Rate $g\ m^{-2}\ d^{-1}$	PPF Utilization Efficiency $g\ m^{-2}\ d^{-1}\ mol^{-1}$	Leaf Area Index	Shoot Percent Dry Mass
400	23.0	30.3	1.32	13.4	10.9
600	34.6	44.2	1.28	13.5	10.7
800	46.1	46.1	0.98	14.2	11.2
1000	57.6	43.9	0.76	15.7	11.2
1400	80.6	53.9	0.67	15.4	11.7
1700	103.7	65.5	0.63	18.9	11.8

* Plants harvested at canopy closure, 24 days after planting
Plant density: 2,000 plants per m^2
Cultivar: Yecora Rojo

plants per m^2, but it still required the labor-intensive step of transplanting. We are now using an inert rockwool material (Grodan; used in commercial hydroponics) that allows us to plant seeds directly at densities of up to 10,000 plants m^{-2} or higher. The rockwool material is kept wet during germination and until roots are well established in the nutrient solution below. Optimum density in one preliminary experiment was about 2000 plants m^{-2}.

RESULTS OF MANIPULATING ENVIRONMENTAL AND CULTURAL FACTORS

Optimizing Photosynthetic Photon Flux: Energy, Mass, and Volume Tradeoffs

Energy efficiency may become very important in a CELSS. Energy will be used in many ways in a regenerative system, but energy to power the lighting system for plant growth will be the single largest input if electric lamps are used to provide the photosynthetic photon flux (PPF) to drive photosynthesis. Changes in PPF utilization efficiency by plants could save more energy than all the other CELSS energy inputs combined. CELSS plant growth systems that utilize sunlight directly or that are powered by a nuclear reactor might not need to be concerned with PPF utilization efficiency. In these situations a large energy input could be used to reduce the mass and/or volume of the food production system. We have recently begun PPF input studies that are designed to determine the tradeoffs among energy, mass, and volume input parameters. Table 1 indicates that crop growth rates increase in direct proportion to increases in PPF ($r^2 = 0.85$). Although the growth increase is linear up to the highest PPF level tested, each additional unit of PPF input is used less efficiently by the plant canopy.

Optimizing Plant Nutrition in a Recirculating Hydroponic System

All plants can be grown hydroponically with a few basic nutrient solution recipes. There is a big difference, however, between slow growth and highly optimized, rapid growth. Achieving optimum growth and nutrition from seed imbibition to physiological maturity requires nutrient solutions that are individually tailored for each species being grown. The optimum composition of these solutions can change during the plant's life cycle and may need to be altered for different photosynthesis/transpiration ratios.

Very little research has been published on optimum concentrations of nutrients in solutions for wheat. When standard nutrient solutions are used for wheat, nutritional deficiencies or imbalances have appeared in our growth conditions. These imbalances are not always severe, but they can cause such foliar symptoms as necrotic leaf tips. We have observed that nutrient uptake in our circulating solution systems is not the same as in other, aerated but stationary containers. We have also observed that foliar symptoms vary with different transpiration rates, which are altered by leaf/air vapor-density gradients and by stomatal apertures, which are, in turn, altered by CO_2 levels. Table 2 shows the composition of nutrient solutions used during a life cycle of wheat in several of our successful yield trials. Note that ammonium ion is not added in the original solution but only in the

TABLE 2 Nutrient-Solution Compositions (mmoles per liter)

Mineral	Initial	Make-up
NH_4^+	0.0	0.01*
NO_3^-	15.0	3.75
P	0.2	0.5
K	3.2	1.00
Ca	12.0	1.50
Mg	4.0	0.50
S	2.0	0.50
Cl	16.0	0.04
Fe	0.124	0.0125
B	0.080	0.020
Mn	0.008	0.002
Zn	0.0008	0.0002
Cu	0.0003	0.000075
Mo	0.0001	0.000025
Si	0.300	0.075
Na	0.600	0.15

*NH_4^+ is added in pH control solution; 0.01 mM is an approximate average concentration.

in the make-up solution. Silicon may not be essential for wheat growth, but it is possible that lodging is less of a problem when plants have ample silicon.

Plants almost always respond to a nutrient deficiency by partitioning more photosynthate into the root system. This reduces the percent edible biomass. In short, optimum growth cannot be achieved by altering only the foliar environment. For this reason, we have built recirculating nutrient solution systems that can provide replicated data on root-zone effects in different foliar environments. We plan to use these, as well as our aerated, individual container systems, to further investigate and refine the root-zone ionic composition. Essential elements and deleterious levels of non-essential elements may both need to be investigated. We currently monitor, by ICP emission spectrophotometry, the concentrations of the essential elements in both foliar tissue and nutrient solutions.

Nitrate/ammonium nitrogen ratios.

Wheat plants absorb ammonium from solution more rapidly than any other ion. Hydrogen ions are secreted to balance uptake of ammonium ions. We have found that this occurs throughout the life cycle. Providing ample ammonium along with nitrate nitrogen in nutrient solution has enhanced total nitrogen uptake in short-term studies of Cox and Reisenauer /1/ and of Huffaker, Reins, and Qualset /2/. An increased nitrogen content of foliar plant parts is associated with increased photosynthetic rates, prolonged leaf photosynthetic output, and increased grain protein. The nitrate/ammonium ratio can also alter the uptake of other ions. This ratio can be easily controlled in solutions, and its long-term effects on wheat growth need to be further studied. We plan to alter the concentrations of these ions in adjacent hydroponic systems, to monitor their uptake rates using our nitrate- and ammonium-specific ion electrodes, and to relate uptake rates to altered plant growth, specifically nitrogen and protein concentrations in foliar plant parts.

The Roles of Photoperiod and Temperature in Yield of Grain

The conditions that promote fastest total growth do not lead to the highest yields. This finding represents a major shift in our research approach. We have developed the term phasic environmental control to indicate that environmental conditions will need to be different for each phase of plant development. It now appears that maximum yields cannot be achieved without phasic environmental control. Five phases of development in the wheat life cycle are as outlined in Table 3.

Warm temperatures (25°C and above) promote rapid growth rates and a short life cycle, but they lead to small wheat spikes and very poor pollination. This is illustrated by Fig. 1 and Table 4. Cooler temperatures (20°C) cause slightly slower growth rates and lengthen the life cycle but greatly increase the spike size and seed set. At the cooler temperatures, a

TABLE 3 Five Stages of Wheat Development and associated length in a 60-day life cycle

Developmental Stage	Day Number	Associated Morphological Change
1. Vegetative	0-12	Germination and early leaf growth
2. Reproductive Initiation	13-18	Microscopic changes in apical meristem that determine ultimate spikelet number
3. Extension	19-30	Further development of floral parts and final determination of florets per spikelet
4. Anthesis	30-35	Pollination and fertilization
5. Grain Fill	36-60	Assimilate translocation into developing seed

TABLE 4 Photoperiod/Temperature Influence on Yield Components in Wheat

Plants per m^2	Spikes per m^2	Total Seeds per Spike	Mass per Seed (mg)	Total Yield $g\ m^{-2}$	Days to Harvest	Yield $g\ m^{-2}\ d^{-1}$	Harvest Index %	Yield $g\ m^{-3}\ d^{-1}$	$g\ m^{-3}\ d^{-1}\ mmol^{-1}$
Cool Temp. (20°C day, 15°C night), 14-h photoperiod									
1150	2007	21	29	1154	77	15.1	46	16.3	323
Cool Temp. (17°C), 24-h photoperiod									
1076	2387	16	30	1054	66	16.3	35	17.5	203
ns	ns	*	ns	ns	*	ns	*	ns	*
Warm Temp. (27°C), 14-h photoperiod									
1030	3234	5	27	279	66	4.3	11	4.3	85
Warm Temp. (27°C), 24-h photoperiod									
830	2128	10	29	872	61	14.2	29	14.4	167
*	*	*	ns	*	*	*	*	*	*

Statistics: Duncan's Least Significant Difference Test ($\alpha = 0.05$);
* = significantly different, ns = not significantly different.

shorter photoperiod also increased the number of seeds per spike and the yield per meter; but the days to harvest were also increased, so yield per day was decreased. In other experiments, however, we have also observed increased daily yields in response to relatively short photoperiods. These temperature and photoperiod effects are an example of the need for phasic environmental control to obtain the best of both worlds.

TESTING GERMPLASM AND BREEDING PLANTS FOR CONTROLLED ENVIRONMENTS

Results of Greenhouse Studies

We have tested about six hundred cultivars, made several hybrid crosses, and have begun large scale testing with our most promising breeding lines (Tables 5 and 6). These tests require considerable area, and it is not feasible to conduct them in our growth chambers. The tests, have, therefore, been conducted in a greenhouse section that provides environmental conditions similar to those that promote highest yields in the growth chambers.

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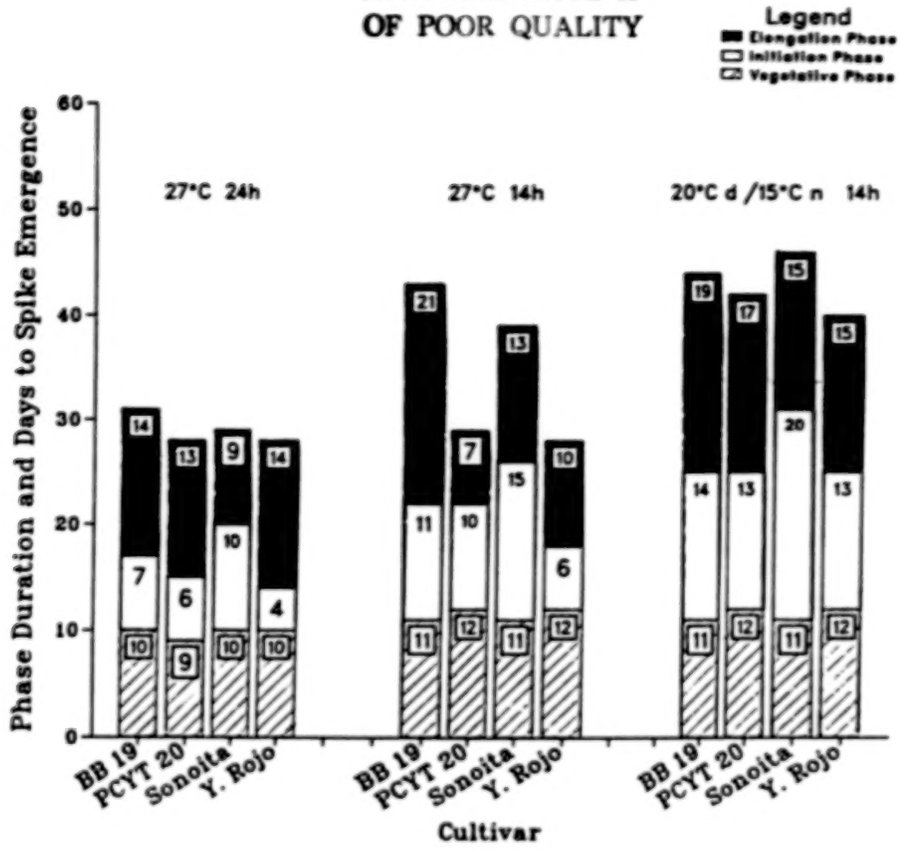

Fig. 1. Temperature/photoperiod influence on the phasic development of wheat.

TABLE 5 Results of a Replicated Cultivar Evaluation in the Greenhouse

Cultivar	Yield g m^{-2}	Ht cm	Yield g m^{-2}	Seeds per Head
BB-8	711	51	783	37
Veery 10	610	46	707	31
BB-16	548	38	692	40
PCYT 20	649	68	603	31
Fielder	664	88	519	33
Yecora Rojo	504	58	518	24
Sonoita	511	60	512	27
Anza	602	89	467	35
Fremont	541	86	428	35
BB-19	342	41	419	47
BB-11	375	53	406	35
Y.ROJO x O.Dwf.	328	47	376	15
Olesen's Dwarf	186	30	261	8
DLSD*	115.5	5.1	129	11

*Mean separation by Waller-Duncan test, K ratio = 100

We now have a group of 9 homozygous lines that are short (33 to 50 cm tall), have excellent head size and seed set, but may have below-average mass per seed at harvest. Good head size and seed set are much more difficult to achieve with environmental modifications than good

TABLE 6 Results of a Replicated Uniculm Trial in the Greenhouse

Line ID	Average Seed Mass per Primary Head (g)	Primary Head Seed Mass as Percent of Total Seed Mass
9	2.6	63
18	2.4	77
20	2.3	45
8	2.2	97
2	2.1	50
4	1.9	60
Fremont	1.9	26
12	1.8	63
10	1.8	76
5	1.8	92
1	1.7	97
19	1.5	75
6	1.5	100
Uniculm	1.4	95
DLSD	0.87	29

TABLE 7 Cultivar Evaluation: Replicated Study in Controlled Environments

	Spikes Per m^2	Seeds Per Spike	Mass Per Seed (mg)	Yield $g\ m^{-2}$	Days To Harvest	Yield $g\ m^{-2}\ d^{-1}$	Harvest Index %	Height cm	Yield $g\ m^{-2}\ d^{-1}$
Yecora Rojo	2130	18	34	1224	67	19	47	56	19.2
BB-19	1771	24	23	987	76	13	46	40	16.2
PCYT-20	2641	15	31	1127	71	16	33	65	15.3
Sonoita	1562	21	32	1067	76	14	50	56	14.6
DLSD ($\alpha = 0.05$)	*	*	*	*	*	*	*	*	*

grain fill, so we are none-the-less very excited by this genetic contribution. We are also working with uniculm lines (Table 6) that could be density planted, leading to rapid canopy formation.

The most significant message from our plant breeding efforts is that additional genetic selection is likely to have major effects on food production in a CELSS environment.

Results of Controlled-Environment Studies

Early breeding trials in the greenhouse indicated that line BB-19 was the most promising line, so it was selected for more detailed testing in controlled environment studies. Table 7 shows the mean values for yield components from three high yielding environments. Yecora Rojo, PCYT 20, and Sonoita are daylength-insensitive, full-dwarf cultivars (50 to 60 cm tall) that have consistently been high yielding in past studies.

The BB-19 line had significantly more seeds per spike than the other lines, but this did not result in a correspondingly higher yield, because the mean mass per seed was less than other lines. It is not yet clear why the mass per seed is below normal. It is

possible that our ultradwarf lines have such a large head in relation to their leaf area that they have become source limited even in our CO_2-enriched, optimizing environments.

A high yielding cultivar is not useful in a CELSS unless it can develop its high yield in a short period of time. Yecora Rojo was the earliest of the cultivars in this study, and it thus has an excellent yield per unit time. It's harvest index, as well as that of two other cultivars, is close to 50%, as good or better than normally occurs in the field (40 to 45%).

We have focused attention on ultra-dwarf cultivars for the following reasons:

1. Most importantly, they tend to have a higher harvest index than taller cultivars. In this study the tallest cultivar, PCTT 20, had the lowest harvest index. Harvest index is of particular concern in a regenerative system. It has been suggested that ultra-dwarf cultivars are not capable of achieving yields equal to taller cultivars, but this suggestion has been made on the basis of studies in non-optimizing field environments. In these environments the planting density is optimum for tall cultivars but suboptimum for their ultradwarf counterparts. Because of their small size, ultradwarf cultivars require higher planting densities to intercept all the available light.

2. Short cultivars are much easier to work with in the confined spaces of controlled environments.

3. Taller cultivars (50 to 100 cm) require support to prevent lodging. Short cultivars do not require this support.

4. Volume may become an important input parameter in a CELSS.

For these reasons, we are particularly interested in yield as g m^{-3} d^{-1}. The cubic meters of space are determined for the tables by measuring the final height of the cultivar and then adding 0.4 m to this value. The 0.4 m is added as space for the root zone and for the lighting system. A 60-cm cultivar would thus have the same yield per $meter^3$ as it does per $meter^2$. Using this ultimate expression of yield (g m^{-3} d^{-1}), Yecora Rojo was significantly higher than the other three cultivars. We are using it as a standard for our environmental studies.

We are also studying clonal propagation of wheat. The goal of this companion project, under the direction of Dr. John Carman, is to produce many (thousands) of somatic embryos from a single callus culture. Such an approach would allow the use of F_1 hybrids and would allow the astronauts to eat the seeds that would otherwise be needed for the next crop (up to ten percent of the harvest). It would have to be simple enough to be performed by astronaut farmers without too much expenditure of time. So far, the approach looks promising.

CONCLUSIONS

Our research has utilized small, replicated communities of wheat plants rather than individual, spaced plants. This approach requires more space for each treatment but allows us to directly measure treatment effects per unit area and thus to accurately predict the performance of the entire food production system of a CELSS.

The components of yield in wheat (spikes per $meter^2$, seeds per spike, and mass per seed) are directly affected by planting density; thus, optimal environmental conditions vary with density. We therefore feel that simultaneous optimization of both cultural and environmental factors is important to rapid progress in CELSS research.

It is possible to grow wheat through a 70-day life cycle and to obtain high yields in a completely recirculating nutrient solution. The ionic composition of the solution must be maintained, but the organic efflux from the plant roots appears to be rapidly oxidized by the microorganisms in solution. Total organic carbon has been about 10 mg per liter in our systems.

Obtaining rapid growth rates, a short life cycle, and high grain yields may require the use of phasic environmental control, which is the application of different environments at different stages of the wheat-plant life cycle. This type of control might require a CELSS design that had several relatively small compartments rather than one large compartment.

Developing new wheat cultivars for controlled environments may not only be useful but necessary to optimum efficiency in a CELSS.

The problems and potentials of this research are great. We anticipate using our customized controlled environment facilities to focus on obtaining data to determine the feasibility and future design of a CELSS. Although our yields are well above those obtainable in the field, they are still well below what they could be based on photosynthetic and cropping efficiencies. Much progress remains to be made.

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N88-12271

THE EFFECT OF RADIATION ON THE LONG TERM PRODUCTIVITY OF A PLANT BASED CELSS.

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ABSTRACT

Mutations occur at a higher rate in space than under terrestrial conditions, primarily due to an increase in radiation levels. These mutations may effect the productivity of plants found in a controlled ecological life support system (CELSS). Computer simulations of plants with different ploidies, modes of reproduction, lethality thresholds, viability thresholds and susceptibilities to radiation induced mutations were performed under space normal and solar flare conditions. These simulations identified plant characteristics that would enable plants to retain high productivities over time in a CELSS.

INTRODUCTION

Controlled ecological life support systems (CELSS) will form at least part of the overall life support system used for deep space missions /1/. These missions will have a duration of years, a limited crew, and probably severe mass limitations. CELSS designed to operate in space must perform within these constraints as well as those imposed by the space environment.

Plants will probably be used to perform at least some of the functions in a CELSS, including biomass generation, carbon dioxide reduction, oxygen regeneration, and waste recycling /1/. The selection of plant species for use in a CELSS will take into consideration several factors. To minimize CELSS energy, size and mass requirements, the net productivity of the plants to be grown should be maximized with respect to these parameters. The biomass obtained from the plant should meet as many human metabolic requirements as possible. The plant should be capable of being cultivated with little human intervention and should require only a simple growth and harvesting system. The plant must suffer little or no productivity losses due to multiple generation growth in the space environment.

The environment found in space will effect the growth of plants. Microgravity can induce gravitropic effects during the development of the plant /2/. Radiation can cause phenotypic and/or genotypic effects in plants /3-7/. Any CELSS deployed in space will not have large excess capacities for production built into it, as a result of mass and volume restrictions. If any aspect of the space environment reduces the production levels of a CELSS, unscheduled terminations of flight personnel may occur.

This study examines the genotypic effect of radiation on the productivity of a plant based CELSS at space normal levels as well as during solar flare activity.

COMPUTER MODEL DEFINITION

Plants exhibit large variations in structural characteristics, gene numbers, reproductive unit type and weight and other attributes. These characteristic variations were standardized in order to compare the effect of radiation on plants that vary in reproductive mode, ploidy, viability thresholds, lethality thresholds, and susceptibility to mutation at constant radiation levels. Therefore any plant used in the model may not truly represent any one real plant species.

Multi-generation Operations

The model examined the multi-generation effect of radiation induced mutations on the productivity of an isolated, size limited CELSS containing one species of plant. In each generation a maximum of 100 plants were grown. A limited plant number was selected to ensure that both general trends in system productivity and the skewing effect of random events could be detected. Each plant could produce an upper bound of 10 reproductive units (RUs). This number represents a compromise between the usually smaller number of RUs produced by vegetative plants and the usually larger number of RUs produced by seed producing plants. A subset of the RUs produced by these plants were used to start the next generation of plants, with the remainder being used as a food product. Key variables that affect the food product yield were recorded for each plant generation. For each generation these included : the number of plants grown (max 100), the total number of RUs produced (max 1000), net system productivity (max 900), number of RUs that were germinated (max 100), the number of RUs that failed to germinate, the number of germinated RUs that reached RU production, and the number of gene mutations that occurred.

A majority of the model variables were assigned static values for the entirety of a multi-generation run. Configuration choices included the modelled plant's (MPs) ploidy, reproductive mode, reproductive selection criterion, lethality threshold, viability threshold, genes subject to mutations, and frequency of gene mutation outcomes. The model assumed that productivity, viability, and lethality were under genetic control only and were not subject to phenotypic variations. Mutation frequency could be altered between generations to reflect solar flare conditions, although a constant mutation rate would be typical for most situations. The plants used for generation zero normally contained fully functional (wild type) genes, although specific genetic deficiencies could be induced before beginning a multi-generation run.

Modelled Plant (MP) Characteristics

MPs contained genes that express only three traits considered essential for food production. Productivity measured total food product produced over time by plants that reach productive status. Genes that reduce RU weights, RU number, or biomass weight and increase growth periods were considered to reduce productivity. Productivity was measured as the RU number, assuming constant RU weight, with a MP producing a maximum of 10 RUs. Viability measured the proportion of RUs produced that were capable of germinating. Genes affecting viability reduced germination frequencies. Lethality measured the proportion of plants after germination that would die prior to reaching adulthood due to gene dysfunction. Genes affecting lethality were considered to be those that kill the plant or simply prevent it from producing food.

A MP contained 60 genes per chromosome, with the three MP genetic traits being represented by 20 genes per chromosome each. These 20 genes were divided into two gene clusters, each composed of four different sized gene systems. Gene systems consisted of one, two, three or four genes, which yields a total of 10 genes per gene cluster. This gene number was selected to ensure that gene duplication effects would occur and that differences between various plant types could be detected. The ploidy of MPs could be varied to be diploid, tetraploid or hexaploid.

MPs could reproduce in one of four different modes; vegetatively, by anther culture, by cross fertilization, or by self fertilization. Some modes are easier to implement than others : anther culture represents a high technology, labor intensive means of reproduction, while cross fertilization may require mechanical assistance due to a lack of insect or wind mediated cross pollination. During sexual reproduction (cross or self fertilization) or anther culture where gametes were required for reproduction, gametes were produced by meiosis with all genes segregating independently. In anther culture reproduction, one gamete was produced for the plant and the RU was produced from two copies of this gamete. In cross fertilization reproduction, the plant chosen to reproduce was paired with another plant that was randomly or selectively chosen. A gamete from each plant was combined to produce the new RU. In self fertilization reproduction, two gametes were produced from the same plant, and combined to form the new RU. In vegetative reproduction, RUs produced were clones of the parent plant.

Mutations

Genes in the MPs had four different states. Wild type genes were 100% functional. A leaky mutant gene was 50% functional, but would not impair the performance of other genes in its gene set. A recessive mutant gene was 0% functional, and would also not impair the performance of other genes in its gene set. A recessive dominant mutant gene had its function altered to the extent that it completely inhibited the function of all other genes for this trait. Positive mutations that altered genes so they performed better than wild type genes were not considered.

The software simulated various plant environments through three separate gene mutation control switches. Immunity to mutations was achieved for any gene in a chromosome via a simple mutation on/off switch. This allowed mutations to occur only in a single trait if desired. Mutation frequency was variable for each generation of plants. When a mutation occurred, the type of gene transformation that occurred could be varied in frequency for each of the four states (wild type, leaky, recessive, and recessive dominant.)

RESULTS AND DISCUSSION

The plants considered for use in a CELSS are in many cases available in different ploidies or may reproduce by a variety of reproductive modes. Genotypes within a species may also exhibit different levels of mutations under equal radiation flux conditions. In these cases, and in cases where different plant species may be used in a CELSS, computer simulations can aid the selection of plant types that will tend to suffer the least losses in net system productivity due to radiation induced mutations. For all simulation runs described here, the mutation rates were increased until differences, if any, between differing plant systems were noted.

Nonflare Conditions

In nonflare conditions the radiation flux encountered in space can be expected to be relatively constant. Space missions that are performed in periods of low solar activity can therefore expect relatively constant mutation rates to occur in each cultivar of the plant species used in a CELSS.

The effect of variation in mutation frequency on net system productivity in nonflare conditions.

The mutation frequency that a particular genotype will experience under nonflare conditions is important in determining whether or not it should be selected as a potential plant in the CELSS used. For all types of reproduction, regardless of ploidy, increasing the mutation frequency experienced by the plant had a negative effect over time on net system productivities (Figure 1).

The effect of ploidy on net system productivity in nonflare conditions.

Some plant genera, for example Triticum (wheat), contain species that have different ploidies. Lines with different ploidies can also be generated by tissue culture techniques. Under conditions of high system mutation frequencies, increasing ploidy for plants reproducing by any type of reproduction resulted in higher retained net system productivities over time (Figure 2). At lower

mutation frequencies the differences between the net system productivities of plants reproducing by the same means with differing ploidy declined or disappeared (Figure 3).

Th effect of reproduction mode on net system productivity in nonflare conditions.

Various plant species can reproduce by a number of modes of reproduction, both natural and man controlled. The mode of reproduction selected for a plant could affect its net system productivity over time. The effect of varying reproduction modes at a constant mutation frequency were pronounced in this study. High ploidy plant systems utilizing modes of reproduction that select out recessive and leaky mutant genes with higher efficiencies (vegetative and cross fertilization) retained highest net system productivity (Figure 4). Lower ploidy plant systems in vegetatively reproducing plants retained higher net system productivities than cross fertilizing systems and self fertilizing systems retained higher net system productivities than anther cultures (Figure 5). Figure 5 also demonstrates the effect of limited closed systems and randomness on the effect of mutation on net system productivity. In general, cross fertilizing plants retained higher net system productivities than self fertilizing plants. However in this specific case (Figure 5) a random event skewed the net system productivity of one or the other of the plants utilizing these reproduction modes. This has important implications for predicting events in a CELSS. Limited closed systems are susceptible to random events and events like this should be accounted for when planning system production capacity.

The effect of viability thresholds on net system productivity in nonflare conditions.

The viability threshold of a plant affects the ability of a RU to germinate. At viability levels below the viability threshold, a RU will not germinate. This is a reflection of system complexity and gene redundancy. More complex systems have higher viability thresholds due to the higher probability of mutations disrupting the germination process. Systems with higher gene redundancies have lower viability thresholds. Mutations affecting RU germination tend to be selected out of the system as they manifest themselves. If a RU does not germinate, another RU can be selected to take its place because of the short time frame involved. Hence the number of plants initially found in the system is not generally affected by low or medium levels of RU viability. Losses in net productivity occur by the loss of RUs which failed to germinate, unless the RU is not a food product. Such was the case in this study with plants reproducing by anther culture. In all other plant systems, net system productivity declined with higher viability thresholds (Figure 6). These systems represent those with more complex germination processes. This loss of net productivity was due to RUs that failed to germinate (Figure 7). The effect was less pronounced at higher ploidy (and hence higher levels of gene redundancy) with fewer RUs failing to germinate for the reproduction modes affected (Figure 8).

The effect of lethality thresholds on net system productivity in nonflare conditions.

Lethality thresholds affect the ability of a germinated RU to reach productive status. At lethality levels below the lethality threshold, a germinated RU will not reach productive status. In a fashion analogous to viability thresholds, this is a reflection of system complexity and gene redundancy. Unlike viability threshold effects, the effects of lethality thresholds appear after the total plant number has been fixed. These effects are not correctable by simply regrowing another plant to replace the expired plant, as the affects occur well into the process of growth. Plants with higher lethality thresholds for all reproductive modes had lower net system productivities over time as compared to those with lower thresholds (Figure 9). Higher ploidies demonstrated less net difference in net system productivities between plants with different lethality thresholds.

The Effect Of Solar Flares

During periods of increased solar activity, the radiation fluxes encountered by a CELSS will increase the mutation rate of plants found in a CELSS for short periods of time. While space vehicle inhabitants will most likely have some place of refuge that is adequately shielded, the size of CELSS units will make shielding unlikely due to mass restrictions.

The effect of flares without selectivity.

Solar flares occur infrequently and with large variations in magnitude. They are characterized by short term increases in radiation that then subside back to nonflare levels after a period of time. In these simulations, flares were assumed to affect one generation only. Examination of situations where flares occur revealed that for all reproductive modes at any ploidy, flares resulted in immediate short term reductions in net system productivity followed to different degrees by some level of recovery. The productivity of plant systems after recovery from flares usually eventually equaled the net system productivity of plant systems operating in conditions where flares did not occur (Figure 10). However the short term loss of net system productivity due to flares would in most cases cause a system failure with its associated results.

The effect of ploidy on net system productivity after flares.

As described above all systems experience net system productivity losses in the generations immediately following a flare. Plant systems with higher ploidy suffered smaller initial declines in net system productivity, and recovered more rapidly and generally to a higher net system productivity than plants with lower ploidy (Figure 11). This recovery is not surprising since the chances of producing simultaneous mutations at the same loci should be lower in plants with higher ploidies.

The effect of reproduction mode on net system system productivity in a flare situation.

Similar trends in net system productivity when varying reproduction mode were noted as without flares (Figure 12). Flares however caused large net system productivity losses immediately after the flare. These initial net system productivity losses were not found in nonflare conditions to the same extent.

Selectivity Effect On Retention Of Net Productivity

Selective breeding practices can be employed in closed systems to select for reproduction only those plants that have some high predetermined level of productivity. In all the cases described above no selection was employed in determining which plants were allowed to reproduce. Systems in which some level of selectivity is introduced must have some form of highly automated selection procedure introduced in addition to the automation probably required to operate the CELSS. For this extra automation to be justified clear benefits of selective breeding must be realized.

The effect of selectivity on net system productivity in systems employing various reproduction modes in constant radiation (nonflare) conditions.

Selecting during reproduction only those plants with known high productivity and excluding those with measurable poor productivity may be a way of combating net system productivity declines due to the effects of radiation. Whether selection occurs or not and the degree to which selection is employed will both affect the net result. In general all plants of any ploidy reproducing asexually by vegetative or anther culture means retained higher levels of net system productivity by employing any level of selectivity. The retention of net system productivity in these cases varied directly with the degree of selectivity employed (Figure 13). Plants of any ploidy reproducing by sexual means (self or cross fertilization) only had net system productivity enhanced by high degrees of selectivity. Lower levels of selectivity had no advantage over nonselective reproduction (Figure 14). Note that in all cases selection of breeding stock had no effect on system productivity when very high mutation rates prevailed throughout the lifetime of the system (Figure 15).

Flares and selectivity.

By being highly selective between generations, the recovery from flares was enhanced greatly over nonselected or partially selected plant systems (Figure 16).

CONCLUSION

The effect of the space environment on the multi-generational propagation of plants is unknown. However, with respect to the effect of radiation on net system productivity of a plant based CELSS, the selection of plants with ploidies, reproduction modes, and genotypes that minimize potential effects would be advised. To produce a more accurate prediction of the performance of a specific plant in a space based CELSS, more detailed computer models are recommended. A model of a specific plant species should be supported with actual data from a limited multi-generation experiment of the plant being exposed to radiation conditions similar to those found in space.

ACKNOWLEDGMENTS

The authors would like to thank T. Chen, M. Lazar, P. Nagarajan, and D. Ward for helpful comments regarding the manuscript.

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Figure 1 Net productivity versus plant generation for plants reproducing at different mutation frequencies. Self fertilization reproduction mode. Diploid plants. Symbols and abbreviations: TRIANGLE = mutation frequency of 0.1; STAR = mutation frequency of 0.01; SQUARE = mutation frequency of 0.001; and DIAMOND = mutation frequency of 0.0001.

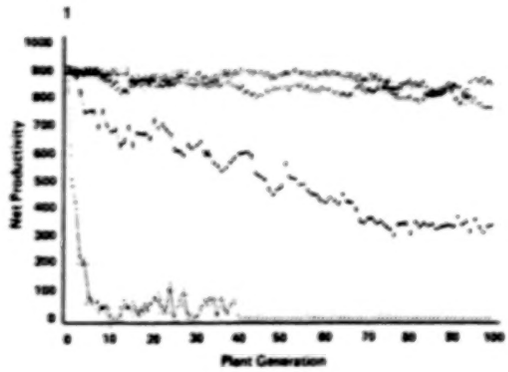


Figure 2 - Net productivity versus plant generation for plants of differing ploidy. Mutation frequency = 0.1. Vegetative reproduction mode. Symbols and abbreviations: TRIANGLE = diploid; STAR = tetraploid; and SQUARE = hexaploid.

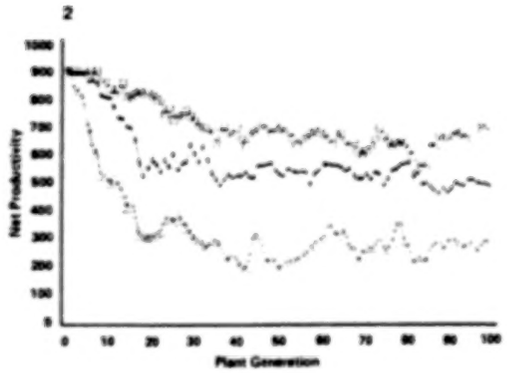


Figure 3 - Net productivity versus plant generation for plants of differing ploidy. Mutation frequency = 0.01. Self fertilization reproductive mode. Symbols and abbreviations as in Figure 2.

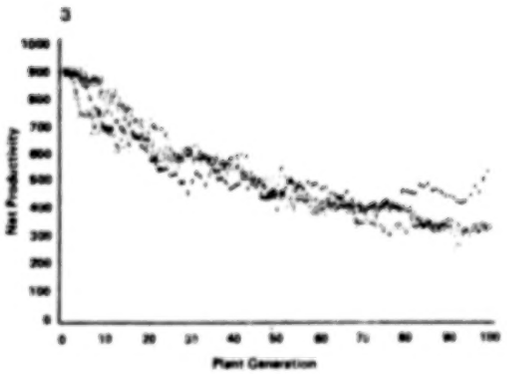


Figure 4 - Net productivity versus plant generation for plants using different modes of reproduction. Mutation frequency = 0.01. Hexaploid plants. Symbols and abbreviations: TRIANGLE = vegetative reproduction; STAR = anther culture; SQUARE = self fertilization; and DIAMOND = cross fertilization.

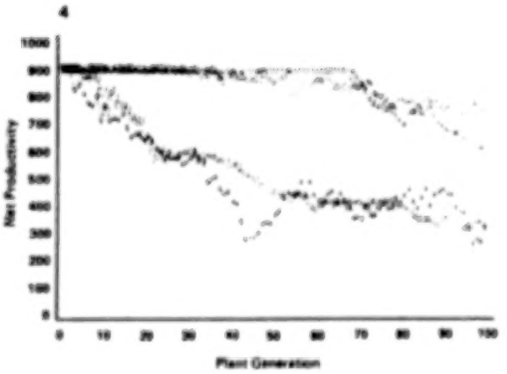


Figure 5 - Net productivity versus plant generation for plants using various modes of reproduction. Mutation frequency = 0.01. Tetraploid plants. Symbols and abbreviations as in Figure 4.

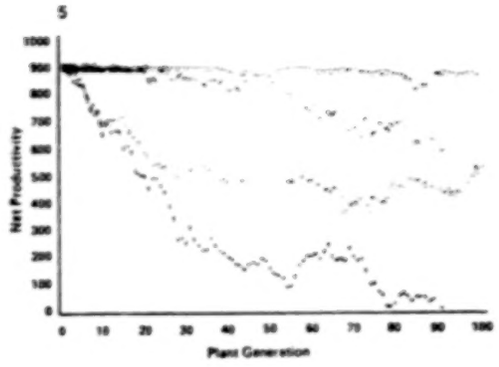


Figure 6 - Net productivity versus plant generation for plants with differing viability thresholds. Mutation frequency = 0.1. Cross fertilization reproductive mode. Diploid plants. Symbols and abbreviations: TRIANGLE = viability threshold of 0.2; STAR = viability threshold of 0.4; SQUARE = viability threshold of 0.6; and DIAMOND = viability threshold of 0.8.

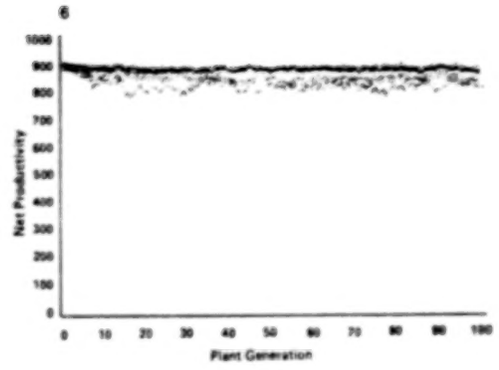


Figure 7 - RUs failing to germinate versus plant generation for plants with differing viability thresholds. Mutation frequency = 0.1. Cross fertilization reproductive mode. Diploid plants. Symbols and abbreviations as in Figure 6.

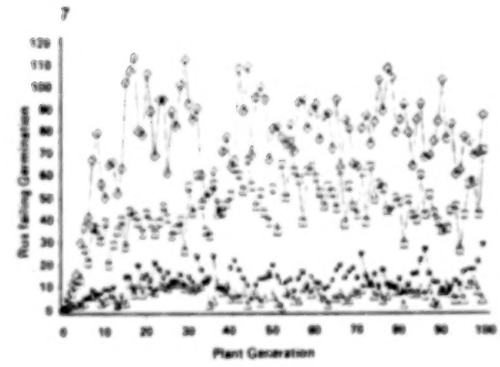


Figure 8 - RUs failing to germinate versus plant generation for plants with differing ploidy. Mutation frequency = 0.1. Cross fertilization reproductive mode. Viability threshold = 0.4. Symbols and abbreviations as in Figure 2.

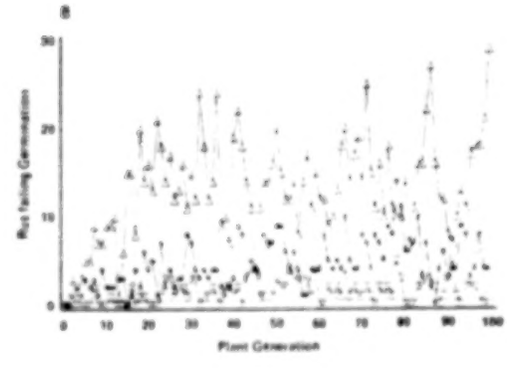


Figure 9 - Net productivity versus plant generation for plants with different lethality thresholds. Mutation frequency = 0.1. Diploid plants. Cross fertilization reproduction mode. Symbols and abbreviations: TRIANGLE = lethality threshold of 0.2; STAR = lethality threshold of 0.4; SQUARE = lethality threshold of 0.6; and DIAMOND = lethality threshold of 0.8.

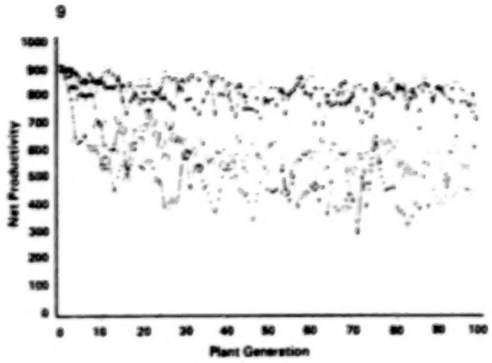


Figure 10 - Net system productivity versus plant generation for plants under conditions with or without solar flares. Cross fertilization reproduction mode. Diploid plants. Mutation frequency for plants under conditions of a solar flare for first generation = 0.5, for generation 2-100 = 0.01. Mutation frequency for plants without solar flares = 0.1. Symbols and abbreviations: STAR = without flare; and TRIANGLE = with flare.

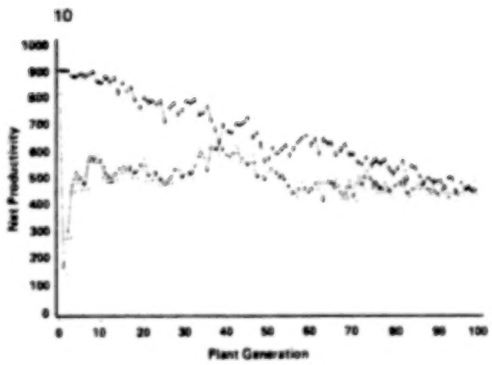


Figure 11 - Net productivity versus plant generation for plants with differing ploidy under conditions of solar flares in the first generation. Mutation frequency in generation 1 = 0.5, mutation frequency for generations 2-100 = 0.01. Self fertilization reproductive mode. Symbols and abbreviations as in Figure 2.

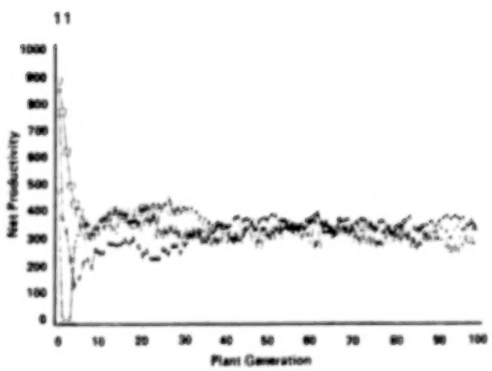


Figure 12 - Net productivity versus plant generation for plants using various modes of reproduction under conditions of solar flares in the first generation. Mutation frequency in generation 1 = 0.5, mutation frequency for generations 2-100 = 0.01. Hexaploid plants. Symbols and abbreviations as in Figure 4.

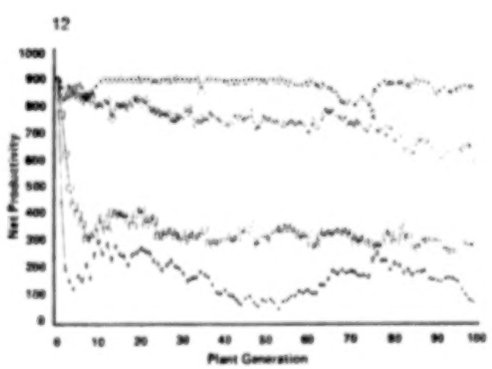


Figure 13 - Net productivity versus plant generation for plants reproducing with different levels of system selectivity during reproduction. Vegetative mode of reproduction. Diploid plants. Mutation frequency = 0.01. Symbols and abbreviations: TRIANGLE = selectivity level of 0; SQUARE = selectivity level of 5; STAR = selectivity level of 10.

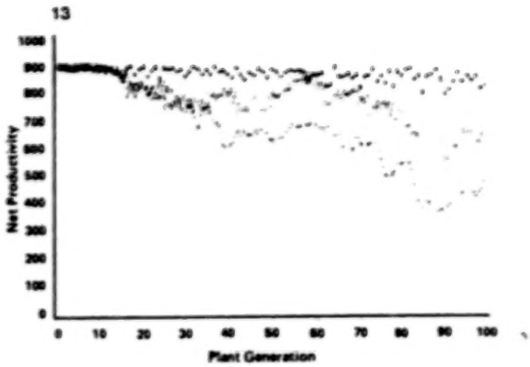


Figure 14 - Net productivity versus plant generation for plants reproducing with different levels of system selectivity during reproduction. Self fertilization mode of reproduction. Diploid plants. Mutation frequency = 0.01. Symbols and abbreviations as in Figure 13.

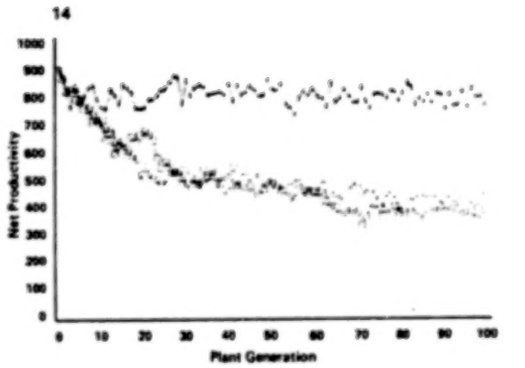


Figure 15 - Net productivity versus plant generation for plants reproducing with different levels of system selectivity during reproduction. Cross fertilization mode of reproduction. Hexaploid plants. Mutation frequency = 0.25. Symbols and abbreviations as in Figure 13.

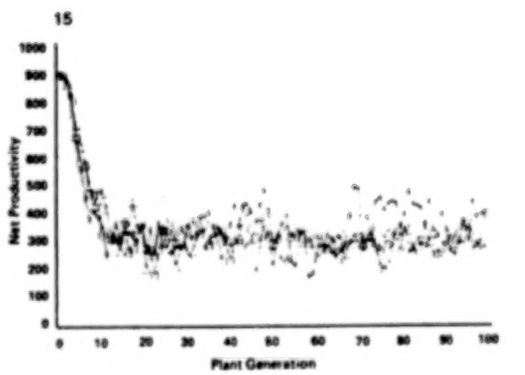
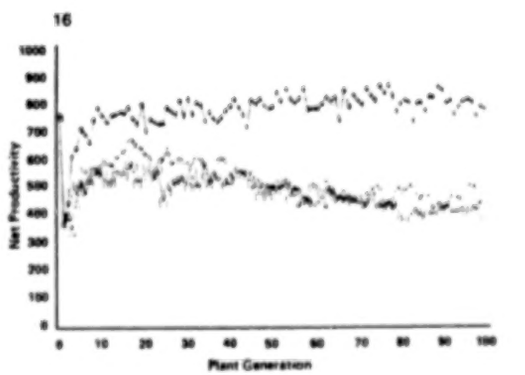


Figure 16 - Net productivity versus plant generation for plants reproducing with different levels of system selectivity during reproduction under conditions of solar flares in the first generation. Vegetative mode of reproduction. Diploid plants. Mutation frequency in generation 1 = 0.5, mutation frequency for generations 2-100 = 0.01. Symbols and abbreviations as in Figure 13.



N88-12272

MASS BALANCES FOR A BIOLOGICAL LIFE SUPPORT SYSTEM SIMULATION MODEL

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ABSTRACT

Design decisions to aid the development of future space-based biological life support systems (BLSS) can be made with simulation models. Here we develop the biochemical stoichiometry for 1) protein, carbohydrate, fat, fiber, and lignin production in the edible and inedible parts of plants; 2) food consumption and production of organic solids in urine, feces, and wash water by the humans; and 3) operation of the waste processor. Flux values for all components are derived for a steady-state system with wheat as the sole food source. The large-scale dynamics of a materially-closed (BLSS) computer model is described in a companion paper /1/. An extension of this methodology can explore multi-food systems and more complex biochemical dynamics while maintaining whole-system closure as a focus.

INTRODUCTION

Until actual closed systems with humans are built and tested, mathematical models utilizing empirical knowledge of components will be the only means available to test hypotheses regarding the operation of these systems, particularly regarding those properties characteristic of the system-as-a-whole. Such models have been important in the research towards biological life support systems /2-4/. Averner /2/ developed the concept of elemental balanced here extended to generalized stoichiometry. Others /3,4/ investigated the influence of feedback controls on recovery following a waste processor failure, illustrating the potential for complex whole-system dynamics. Our intent is to simulate a system that embodies current understandings of space station module volumes, experimental plant growth results, human metabolism, etc., to produce a model that provides information regarding the flow dynamics of a space-based BLSS.

In a companion paper /1/ we develop a mathematical simulation of a closed system that will be able to approach whole-system issues, such as the need for reservoirs and buffers /5/. Biological models at the ecosystem level /6/, the whole-person level /7/, the whole-plant level /8/, the plant-part (leaf) level /9/ are appropriate for other specific inquiries. An awareness that different structural/functional levels possess different properties that may not be obvious from any other level /10/ is essential. Therefore our focus is on the whole system for potential applications for space life support. This system is constructed from major components, such as the plant growth module, the crew compartment, and the waste and environmental controls, each of which are in turn complex systems with internal dynamical parts. This paper examines these systems on the biochemical level in order to develop stoichiometric relations governing the material flows in the system model /1/, focusing on the elements that constitute most of the system's mass: carbon (C), hydrogen (H), oxygen (O), and nitrogen (N). More complex processes can be incorporated in the future, but rather than constructing any particular component in great detail, our aim is to first construct an entire operational model system that is capable of further and diverse modification.

THE COMPONENTS

The Plants

The three general categories of food type -- proteins, carbohydrates, and fats /11/ -- are metaboloids. Although a vast plethora of substances are manufactured by plants, we take the triad of protein, carbohydrate, and fat as important substance-types to track in the model. This necessitates plant species-specific equations that convert carbon dioxide (CO_2), water (H_2O), and a nitrogen source into these food types. These equations are now developed, along with reasons for chosing particular canonical formulas, which are summarized in Table 1.

Carbohydrate. Photosynthesis, most simply expressed, creates glucose and free oxygen from carbon dioxide and water by

$$6\,CO_2 + 6\,H_2O \dashrightarrow C_6H_{12}O_6 + 6\,O_2 \qquad (1)$$

This reaction has a Gibb's Free Energy change of +686 kcal /12/ and therefore $C_6H_{12}O_6$ contains energy that can be used to drive other reactions in the plant. Glucose, the most abundant monosaccharide, is the parent from which most others are built, including the much larger polysaccharides /12/.

Protein. To obtain a representative canonical protein, the 20 amino acids present in most proteins /13/ are first averaged by calculating the CHON mass fractions for each amino acid and then averaging the twenty C-fractions, H-fractions, etc. For this procedure to be perfectly valid, the 20 amino acids should be equally abundant in terms of mass in proteins, which is not the case for any particular protein, but is reasonable for a range of proteins over a range of organisms. The average obtained is listed in Table 1 as "mass av'd amino acid". Another possible way of averaging is to assume the different amino acids are present in proteins in approximately equal numbers; this average is obtained by summing the C-atoms, H-atoms, etc. in all 20 amino acids and then dividing by twenty. This method results in " no. av'd amino acid" in Table 1, with its CHON fractions very similar to those from mass-averaging.

A simple formula that approximates these empirical element distributions is $C_4H_7O_2N$, shown in Table 1. Proteins, in turn, are formed by chaining amino acids through peptide bonds /13/, which effectively removes an H_2O from each amino acid. Therefore, rather than using the average amino acid as protein, a protein formula is better represented by C_4H_5ON, which gives a N-content close to the 16% found in typical proteins /11/. Therefore the reduction of nitrogen from nitrate to ammonia and its incorporation into protein during biosynthesis from carbonhydrate (hexose) is written

$$C_6H_{12}O_6 + 3\,HNO_3 \rightarrow 6\,CO_2 + 3\,H_2O + 3NH_3 \qquad (2)$$

$$2\,C_6H_{12}O_6 + 3\,NH_3 \rightarrow 3\,C_4H_5ON + 9\,H_2O \qquad (3)$$

Equations (2) and (3) can be added,

$$C_6H_{12}O_6 + HNO_3 \rightarrow C_4H_5ON + 2\,CO_2 + 4\,H_2O \qquad (4)$$

Fat. The category fat is taken here to include all substances in the ether extract of a Weende analysis /11/, for example, fatty acids, waxes, and phosphoglycerides. They are all characterized by low amounts of O (and zero N) relative to C and H, and are therefore similar in structure. Palmitic and stearic are most common saturated fatty acids found in both animal and vegetable fat /14/. Palmitic ($C_{15}H_{31}COOH$) will be taken as the canonical fat with CHON fractions listed in Table 1 and formed by biosynthesis from carbohydrate by

$$8\,C_6H_{12}O_6 \rightarrow 3\,C_{16}H_{32}O_2 + 21\,O_2 \qquad (5)$$

The energy needed to form fat and any other plant product is not explicitly accounted for in these equations, for the reverse reaction of equation (1) or similar respiration processes would have to occur along with equation (4). Respiration does not contribute to the mass of the plant, which is the focus here, but is important in the system simulation /1/.

Fiber. Plant fiber is important in a simulation model because there will be inedible parts of the plant, primarily because of the presence of large, linked polysaccharides that inhibit digestibility /11/. These are usually considered separately from the even less digestible lignins (see below). Although the fibers have a complex biochemistry that includes hemicelluloses, celluloses, and xylans /15/, cellulose as the basic structural material of cell walls in all higher land plants will be the representative fiber, with formula $(C_6H_{10}O_5)_n$ /11,16/

$$C_6H_{12}O_6 \rightarrow C_6H_{10}O_5 + H_2O \qquad (6)$$

Lignin. The final important plant component is taken to be lignin because of its ability to appreciably reduce digestibility in small amounts /11/ and is usually found associated with the fibrous materials. A typical lignin is characterized by chains of $C_{10}H_{11}O_2$ (11) and can be formed by

$$20\,C_6H_{12}O_6 \rightarrow 12\,C_{10}H_{11}O_2 + 54\,CO_2 + 21\,H_2O \qquad (7)$$

The Humans

Respiration and waste solids. Humans consume food containing protein, carbohydrates, and fats defined by the same canonical formulas produced by the plants. A fraction of the carbohydrates and fats are assumed to be completely respired to form CO_2 and H_2O by reversing equations (1) and (4). Humans also produce organic solid wastes of different types than found in foods. These -- urine, feces, and wash water solids -- will now be considered.

Urine solids. Human urine has on the order of 25 organic constituents /17/ whose concentrations each exceeds 50 mg/l. Urea ($(NH_2)_2CO$) is by far the most abundant /12,17/, with well over 50% of the total organic solids, followed by much smaller amounts of creatinine, uric acid, ammonia, and still smaller amounts of phenols and oxalates /18/. The CHON fractions of urea and of urine solids given calculated from Lehninger's listing of compounds /12/ is shown in Table 1; it would appear that urine solids are esentially urea. However, a substantially different distribution with more O and less N appears in the elemental listing measured from a standardized freeze-dried urine samples /19/, which is also shown for comparison in Table 1. We obtained a value for the unlisted O in this reference by subtracting the sum of the 15 elements listed from a total of 100%. The reason for these CHON differences is not apparent.

The most complete listing of compounds and CHON fractions for categories of compounds is that of Putnam /17/, whose total O and N for organic solids are between those values from references /12/ and /19/; see Table 1. This study /17/ notes that the formula $C_2H_6O_2N_2$ approximates the CHON distribution of urine solids. This formula can be re-structured as urea plus a sugar precursor ($(NH_2)_2CO + CH_2O$). Although significant glucose in urine is characteristic of a pathology /18/, the many nitrogenous compounds other than urea that exist in urine and the concentrations of the non-nitrogenous compounds happen to produce the above average urine compound, whose urea percentage of the total is a reasonable 67%. Excretion of urea is a principle method the body has to rid itself of ammonium ions in the blood and tissues, and this urea is formed by the hydrolysis of arginine to ornithine /18/. Since arginine can be synthesized and the pathways are more complex than we need for the BLSS system at this point, we will take the formation of urine solids ($C_2H_6O_2N_2$) from the generalized protein to be

$$2\,C_4H_5ON + 7\,O_2 \rightarrow C_2H_6O_2N_2 + 6\,CO_2 + 2\,H_2O \qquad (8)$$

Feces solids. Table 1 lists the CHON fractions from two sources /16, 19/, in which the latter's O was obtained by difference from the table listing elemental composition of freeze-dried feces. The differences between the two sources is perhaps not too surprising given that feces is almost as varied as the food that is eaten. However, following the procedure for urine solids to include some recognition of the composition, Orten and Neuhaus /18/ note that the dry content of feces is 25-50% dead and living bacteria; bacteria are about 50% protein /20/. Feces is an important export for fats from dietary by-products or undigested lipids, and lipids of various types can constitute up to 33% of the dry mass of feces /18/. Other undigested, indigestible or unabsorbed food residues are found. As a possible feces solids composition we take 50% protein (25% from bacteria, 25% undigested food), 25% fat, and 25% carbohydrate. The carbohydrate is not intended to represent undigested glucose, but rather serve as a proxy for undigested non-nitrogenous plant matter. Using the formulas for protein, fat, and carbohydrate above, this mass distribution is approximated by

$$5\,C_4H_5ON + C_6H_{12}O_6 + C_{16}H_{32}O_2 \rightarrow C_{42}H_{69}O_{13}N_5 \qquad (9)$$

This produces CHON fractions generally between those of the two references and will therefore be taken as reasonable. The concern with the substances that comprise urine and feces solids is motivated by the requirement that the BLSS system model should ultimately contain feedbacks within the system. Ultimately, rather than specifying the amounts of urine and feces solids produced each day, they should be linked to the composition of the food eaten. This will be facilitated by deriving some of the reasons behind the CHON fractions of these solids. The same goes for wash water solids.

Wash water solids. Wash solids consist of residues primarily from the skin's surface during washing, and are listed as sweat solids, an important organic solids constituent to be considered along with urine solids and feces solids in designing space life support systems /21/. The composition of wash water solids has been studied /16/ and is displayed in Table 1, both including and excluding soap from the organics. Since the model is not recycling soap at this point, we will exclude soap (about 30% of the total organics) from consideration. The remaining constituents are lactic acid, urea, glucose, and unknown insolubles. From the analysis and assumptions in reference /16/ about 15% of the total organic solids have the composition of urea and about 85% has approximately the same CHO as $C_6H_{12}O_6$. Following the formulas earlier, this leads to

$$(NH_2)_2CO + 2\,C_6H_{12}O_6 \rightarrow C_{13}H_{28}O_{13}N_2 \qquad (10)$$

Table 1 shows that the CHON distribution of $C_{13}H_{28}O_{13}N_2$ closely reproduces the reference values.

The Waste Processor

The waste processor will receive the both inedible and edible waste plant materials and the human wastes. It will need to handle the edible parts of the plants, for example, if the food supply is too high or is diseased and needs to be scrapped, then material sent to the waste processor will contain some food components. The equations for these processes are essentially various reverse combinations of those above, whereby solids are taken back to CO_2, H_2O, and HNO_3. These will not be repeated here. We assume in the model /1/ that this recycling can be accomplished completely using whatever equipment is necessary, although this need not be a requirement in the future, as the output of actual waste processing tests will be added to the model.

THE SYSTEM

Equations. We now create a system by selective sums of the previously developed equations to form simple balanced transformations for the growth of food and inedible parts by plants, the consumption and respiration of food by humans with the production of wastes, and the recycling back to inorganic forms of all constituents by the waste processor. Specifically, an equation for food production is formed by summing equations (1), (4), and (5). By similarly summing equations (1), (4), (6), and (7) a system for the inedible plant production is obtained. This neglects fats and simple sugars, which are not included here in order to focus on the fiber and lignin components that characterize the inedible portions. The human food equation comes from combining equations (8), (9), and (10) with the reverse of equations (1) and (5). Waste processing the edible parts of plants is the reverse of the growth of food, in other words, summing the reverse of equations (1), (4), and (5); waste processing of the inedible parts is similarly obtained by summing the reverse of equations (1), (4), (6), and (7). Finally, the processing of human waste comes from combining the reverse of equations (1), (4), (5), (8), (9), and (10). These actions lead to the following system:

Growth of edible plant matter:

$$n_1 CO_2 + n_2 H_2O + n_3 HNO_3 \rightarrow$$
$$n_4 C_4 H_5ON + n_5 C_6 H_{12}O_6 + n_6 C_{16}H_{32}O_2 + n_7 O_2 \quad (11)$$

Growth of inedible plant matter:

$$n_8 CO_2 + n_9 H_2O + n_{10} HNO_3 \rightarrow$$
$$n_{11} C_4 H_5ON + n_{12} C_6 H_{10}O_5 + n_{13} C_{10}H_{11}O_2 + n_{14} O_2 \quad (12)$$

Human metabolism of edible plant matter:

$$n_{15} C_4 H_5ON + n_{16} C_6 H_{12}O_6 + n_{17} C_{16}H_{32}O_2 + n_{18} O_2 \rightarrow$$
$$n_{19} C_2 H_6O_2N_2 + n_{20} C_{42} H_{69}O_{13}N_5 + n_{21} C_{13} H_{28}O_{13}N_2 + n_{22}CO_2 + n_{23} H_2O \quad (13)$$

Waste processing of edible plant matter:

$$n_{24} C_4 H_5ON + n_{25} C_6 H_{12}O_6 + n_{26} C_{16}H_{32}O_2 + n_{27} O_2 \rightarrow$$
$$n_{28} CO_2 + n_{29} H_2O + n_{30} HNO_3 \quad (14)$$

Waste processing of inedible plant matter:

$$n_{31} C_4 H_5ON + n_{32} C_6 H_{10}O_5 + n_{33} C_{10}H_{11}O_2 + n_{34} O_2 \rightarrow$$
$$n_{35} CO_2 + n_{36} H_2O + n_{37} HNO_3 \quad (15)$$

Waste processing of human waste:

$$n_{38} C_2 H_6O_2N_2 + n_{39} C_{42} H_{69}O_{13}N_5 + n_{40} C_{13} H_{28}O_{13}N_2 + n_{41} O_2 \rightarrow$$
$$n_{42} CO_2 + n_{43} H_2O + n_{44} HNO_3 \quad (16)$$

Equations (11-16) constitute a system for a biological life support system model if the rate coefficients, n_i in moles per unit time, are known.

A Solution

Equations (11-16) must be individually balanced in order that they operate to change matter between various forms and ensure conservation of species. The 44 unknown rate coefficients, n_i (i = 1...40), are not all independent. For example, since N appears in only one component on each side of equations (11), (12), (14), and (15), $n_3 = n_4$, $n_{10} = n_{11}$, $n_{24} = n_{30}$, and $n_{31} = n_{37}$. As another example, since C appears in one constituent on the left-hand side and in three constituents on the right-hand side of equation (11), $n_1 = 4\,n_4 + 6\,n_5 + 16\,n_6$; similar examples are straightforward. Using this procedure for elemental mass balances, 4 equations can be written for each of equations (11-16), resulting in 24 equations for 44 unknowns. Other constraints must be imposed.

The system is therefore further simplified by specifying constant proportionalities between components in physically-meaningful groupings. For example, as the edible part of the wheat grows, the protein, carbohydrate, and fat components are assumed to remain in a constant set of mass ratios to each other. Similarly with the protein, fiber, and lignin in the inedible portion. Furthermore, the edible and inedible are assumed to develop in constant proportion to each other, which means that the harvest index /22/ fraction of edible-biomass/total-biomass acts as a constant governing proportionality during growth (after a 7 day initial period during which only the inedible grows, see /1/). Also, the protein, carbohydrate, and fat in the food consumed are in the same mass proportions as produced by the plant; the masses of urine, feces, and wash solids maintain constant ratios; the waste processor operates upon the protein, carbohydrate, and fat in the edible and the protein, fiber, and lignin in the inedible in the same proportions as they are produced by the plant, and upon the human waste solids in the same relative proportions as they are produced by the people.

With these assumptions is it possible to satisfy the system of reactions by specifying the above proportionalities and by knowing the plant growth rate, the harvest index, the rate of human food consumption, rates of waste processing of edible matter, inedible matter, and human waste. Objections could be raised to all of these assumptions; for example, the amount of protein in the inedible part of wheat decreases as field wheat develops from grass to hay /11/. But they allow us to begin exploring the dynamics of a BLSS model knowing that an approximately equal level of concern exists in specifying the composition of components. More feedbacks can be added as the model develops.

The dynamics of the full model are given in /1/. At this point we will conclude by looking at the system in steady-state. We specify a diet of 855 gm-wheat/day-person, approximately 3000 kcal/day-person /22/. A harvest index of edible to total mass at time of harvest is taken as 45% from a recent experiment /23/. Other trials have had lower harvest indexes /22/, but the 45% is typical of field wheat. The mass proportions of the edible wheat berry are taken as 16.9% protein, 80.5% carbohydrate, and 2.6% fat from Bugbee and Salisbury /22/ (excluding the 2.6% fiber and 2% ash from their total and re-normalizing the values). These proportions are consistent with other values for the wheat grain /13, 24/.

The composition of the inedible portion is more problematical. We choose here a mix of 35% protein, 50% fiber, and 15% lignin. One analysis of wheat straw has close to 30% lignin with the remainder as fiber /25/. Cereal grass of wheat when 10 cm high has a protein content of 43% and even a significant lipid content of 7% /11/. These protein values are partially maintained up to maturity in the hydroponic experiments /26/, although there is some senescence in the later stages of development towards harvest. The fact that the laboratory wheat is not nitrogen-stressed in the later stages of its growth is the likely reason for higher protein fractions in the inedible parts than found under field conditions. Therefore our inedible values should be considered very tentative. When more biochemical dynamics are added to the model, the effects of various environmental factors on the cycling of elements in the total system can be explored by changing the harvest index and inedible composition.

These values lead to 1.74 moles-N in protein consumed per person per day, which in a steady-state must be excreted in the urine, feces, and sweat solids. We distribute this such that n_{19}=0.55, n_{20} = 0.12, and n_{21} = 0.02 in equation (13). This produces a total urine organic solids of 49.5 gm/day-person, a value generally consistent with a measured value /27/ of total solids of 62.9 gm/day, after subtracting possible values for the percent ash content of total urine solids, 14% /19/, 35% /12/, and 38.2% /16/. The amount of wash organic solids that results from this division is 8.4 gm/day-person, consistent with the 8.8 gm/day-person /16/ after the inorganics and soap are removed from this reference's values. Thus 102.2 gm/day-person is in feces solids, which is substantially higher than the 35.7 gm/day-person found in a 90-day test with highly-processed and easily-digestible foods including meat /27/, but compares better to the 75 gm/day-person for a vegetarian diet /28/.

Using these values, it is possible to compute the molar daily rates for equations (11-16) for a single person. Since the system is in a steady-state, the human eats exactly what the plant consumes, so equation (14) drops away. The results, shown with significant figures that balance the elements to about 0.1%, are as follows:

Growth of edible plant matter:

$$31.29\ CO_2 + 27.81\ H_2O + 1.74\ HNO_3 \rightarrow 1.74\ C_4H_5ON + 3.82\ C_6H_{12}O_6 + 0.086\ C_{16}H_{32}O_2 + 35.39\ O_2 \quad (17)$$

Growth of inedible plant matter:

$$46.59\ CO_2 + 30.22\ H_2O + 4.40\ HNO_3 \rightarrow 4.40\ C_4H_5ON + 3.22\ C_6H_{10}O_5 + 0.96\ C_{10}H_{12}O_2 + 57.08\ O_2 \quad (18)$$

Human metabolism of edible plant matter:

$$1.74\ C_4H_5ON + 3.82\ C_6H_{12}O_6 + 0.086\ C_{16}H_{32}O_2 + 25.14\ O_2 \rightarrow 0.55\ C_2H_6O_2N_2 + 0.12\ C_{42}H_{69}O_{13}N_5 + 0.02\ C_{13}H_{28}O_{13}N_2 + 24.82\ CO_2 + 22.55\ H_2O \quad (19)$$

Waste processing of inedible plant matter:

$$4.40\ C_4H_5ON + 3.22\ C_6H_{10}O_5 + 0.96\ C_{10}H_{11}O_2 + 57.08\ O_2 \rightarrow 46.59\ CO_2 + 30.22\ H_2O + 4.40\ HNO_3 \quad (20)$$

Waste processing of human waste:

$$0.55\ C_2H_6O_2N_2 + 0.12\ C_{42}H_{69}O_{13}N_5 + 0.02\ C_{13}H_{28}O_{13}N_2 + 10.24\ O_2 \rightarrow 6.47\ CO_2 + 5.25\ H_2O + 1.74\ HNO_3 \quad (21)$$

CONCLUSION

The whole-system model /1/ lumps some of the components into large-scale masses while maintaining accounting of the CHON elemental balances: food, plant waste, human waste, as well as CO_2, O_2, H_2O, and HNO_3. The flows of these seven mass-types in the steady-state of equations (17-21) is shown in Table 2. Although an alternative would have been to begin directly with a similar set of mass-types values from the literature and estimate a balance of CHON within this system, there would not have been any internal biochemical consistency in such an approach. It is desirable for a system of mass balances to be readily capable of expansion to include, for example, changes in the edible/inedible ratio and tissue-type during growth, the incorporation of different food plants in conjunction with wheat, such as potatoes, soybeans, and lettuce, and the addition of waste processing components that will deal separately with various parts of the inedible biomass, such as lignin and cellulose, which may be important since a substantial portion of the biomass directly from the plant will be indigestible by humans /29/. By explicitly developing a stoichiometric system and examining reasons behind the composition of mass-types, we have a methodology that can adapt to many of these foreseeable needs.

ACKNOWLEDGEMENTS

This work was supported by: (for T. V.) the NASA-Stanford-ASEE Summer Faculty Fellowship Program and NASA-Ames Joint Research Interchange NCA2-101, and (for J. D. R.) the National Research Council Fellowship Program. We thank R. D. MacElroy for valuable discussions.

TABLE 1 Canonical Formulas and CHON % Composition of Model Constituents

Type and Components	Symbol	C	H	O	N	References and Notes
Edible plant						
Protein	C_4H_5ON	57.8	6.0	19.3	16.9	$C_4H_7O_2N$ minus peptide bond
		47.5	6.9	31.7	13.9	$C_4H_7O_2N$
		47.0	7.5	30.4	15.1	mass av.'d amino acid /13/
		48.3	8.0	28.6	15.1	no. av'd amino acid /13/
Carbohydrate	$C_6H_{12}O_6$	40.0	6.7	53.3	-	assume hexose
Fat	$C_{16}H_{32}O_2$	75.0	12.5	12.5	-	palmitic ($C_{15}H_{31}COOH$)
Inedible plant						
Protein	C_4H_5ON	57.8	6.0	19.3	16.9	see above
Fiber	$C_6H_{10}O_5$	44.4	6.2	49.4	-	represented by cellulose
Lignin	$C_{10}H_{11}O_2$	73.6	6.7	19.6	-	typical formula /11/
Human waste						
Urine solids	$C_2H_6O_2N_2$	26.7	6.7	35.6	31.0	$(NH_2)_2CO+CH_2O$
		27.8	6.1	33.3	32.8	/17/
		20.9	5.8	47.5	25.8	/19/, O by difference
		21.3	7.1	26.2	45.5	p. 840 of /12/
		20.0	6.7	26.7	46.7	urea: $(NH_2)_2CO$
Feces solids	$C_{42}H_{69}O_{13}N_5$	59.2	8.1	24.4	8.2	2:1:1; protein, fat, hexose
		71.1	4.2	12.3	12.3	Table 3-1 of /16/
		46.5	7.3	37.0	9.2	/19/, O by difference
Wash solids	$C_{13}H_{28}O_{13}N_2$	37.1	6.7	49.5	6.7	15%urea, 85%hexose
		40.3	6.3	46.8	6.6	orgs without soap /16/
		54.2	7.9	33.2	4.7	orgs including soap /16/

TABLE 2 Mass Fluxes* for a Steady-State System with Wheat as Food

Mass Type	Plants		Human		Waste Processor	
	In	Out	In	Out	In	Out
CO_2	3426.82	-	-	1092.30	-	2334.52
H_2O**	1044.56	-	-	405.96	-	638.60
HNO_3	387.16	-	-	-	-	387.16
O_2	-	2958.79	804.62	-	2154.17	-
Food	-	855.00	855.00	-	-	-
Plant Waste	-	1044.75	-	-	1044.75	-
Human Waste	-	-	-	161.36	161.36	-

* All values in gm/person-day calculated using integer atomic masses and equations (17-21).

**H_2O values include only that involved in stoichiometric transformations of mass type, which does not include transpiration, drinking water, water associated with wet (as opposed to dry) biomass, etc. See /1/ for these values.

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N88-12273

TRICKLE WATER AND FEEDING SYSTEM IN PLANT CULTURE AND LIGHT-DARK CYCLE EFFECTS ON PLANT GROWTH

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ABSTRACT

Rockwool, as an inert medium covered or bagged with polyethylene film, can be effectively used for plant culture in space station. The most important machine is the pump adjusting the dripping rate in the feeding system. Hydro-aeroponics may be adaptable to a space laboratory. The shortening of the light-dark cycles inhibits plant growth and induces an abnormal morphogenesis. A photoperiod of 12-hr-dark may be needed for plant growth.

INTRODUCTION

In space, we cannot use typical nutrient solution culture systems for plant growth. A new culture system has to be established, e.g. hydro-aeroponics. If the light (l)-dark (d) cycles are 90 minutes (45(l) + 45(d)) as in the flight of space shuttle, abnormal plant growth will be induced. It is important to find effective lighting regimes for plant production in a CELSS. Our research was done to establish a method of rockwool culture and lighting regimes for normal plant growth.

I. TRICKLE WATER AND FEEDING SYSTEM IN PLANT CULTURE

Methodology

Rockwool is made from a mixture of basalt and slag as a by-product of the iron industry. Plant seedlings were grown on rockwool media (7.5 cm high x 10 cm wide x 10 cm deep). After plant roots were sufficiently distributed in these blocks, seedling blocks were placed on rockwool beds (7.5 cm high x 25 cm wide and at least 120 cm long). Rockwool blocks and beds were then bagged with polyethylene sheets. Water and nutrient solution from fert-irrigator were supplied through spaghetti tube inserted into the rockwool bed (Fig. 1).

Results

Rockwool is 3-4% solid phase and 96% fluid phase. Water or solution is dispersed in every direction by capillary attraction among fibers (10 - 100 μm). The total amount of water or nutrient solution to be supplied has to stay within a limitation of 70% of the water holding capacity, because the bed system has no drainage. The water and feeding system are shown in Fig. 2. When the three way electromagnetic valve (EMV) turns on the water, the piston in the pump adjusting the dripping rate is pressed down, and emits the concentrated nutrient solution as a droplet (0.1-5 ml/stroke). After this work, the water is drained away from pump room and nutrient solution is pumped up. In this case, two stock solutions are prepared in order to prevent precipitate formation. The pump adjusting the dripping rate must be a twin-head proportioner type. As the maximum flow rate of water is 0.5 to 1.0 liter per pulse, the stock solution will be diluted 100 - 2,000 times before it finally reaches the plants. This is controlled by a transmitter with a flow meter and a three way EMV via a pump which adjusts the dripping rate. We usually take a 1,000 times dilution of the stock solution to get 40 me/l total concentration. 50 to 200 ml of nutrient solution or water, depending on plant age is provided to an individual plant, as one dose for a few minutes, repeating four to seven times a day by the use of a timer switch.

Fig. 1. Plants in rockwool bed with trickle feed lines.

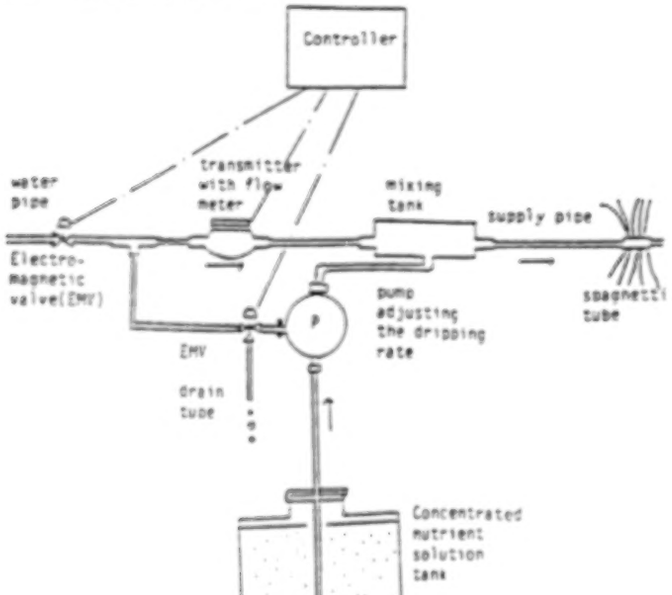

Fig. 2. Main parts of trickle water and feeding system.

Although nutrient composition varies with plant species and its developmental age, the standard ratio of nutrient ions for vegetative growth, i.e. NO_3^-: H_2PO_4: SO_4^{--}: NH_4^+: K^+: Ca^{++}: Mg^{++}, is recommended as 23 : 7.5 : 7.5 : 5 : 20: 15 : 10, percent of total amount (usually 40 or 20 me/l) in chemical equivalents. In reproductive growth stage, SO_4^{--}, Ca^{++}, and sometimes H_2PO_4 have to be increased, whereas NO_3^-, NH_4^+, and K^+ have to be decreased. The minor elements such as Fe, B, Mn, Zn, Cu, and Mo are also required. In the case of rockwool culture, EDTA chelate of a metal is available for the uptake of nutrients, because of the slightly alkaline solution in rockwool medium. pH and electroconductivity (EC) should be adjusted automatically at about 6 and below 2 milimhos/cm, respectively /3,4/. Hydro-aeroponics by the use of fert-irrigation system on rockwool medium may be adaptable to a space laboratory, because it does not require the transport of large amounts of heavy, bulky water and rockwool, as a medium, can be used for several crop cycles.

II. LIGHT-DARK CYCLE EFFECTS ON PLANT GROWTH

Methodology

Komatsuna (Brassica campestris L.) and Mung bean (Vigna radiata R. Wilcz.) were grown for 4 and 3 weeks after sowing, respectively, under 24(LD-12), 12(LD-6), 4(LD-2), 1.5(LD-3/4), 0.5(LD-0.25)-hour cycles with

equal light-dark duration. The light flux rate was adjusted to 175 μmol m^{-2} s^{-1} (400 -700 nm) or 10.6 klux ± 6%. Air temperature was kept at 25 C and relative humidity at about 70%.

Results

All growth parameters measured were reduced with shortening the cycles down to LD-3/4, but more or less increased again toward LD-1/4. In LD-3/4 dry weight and leaf area relative to LD-12 were 46 and 55% in Komatsuna, and 14% and 15% in mung bean, respectively (Fig. 3,4).

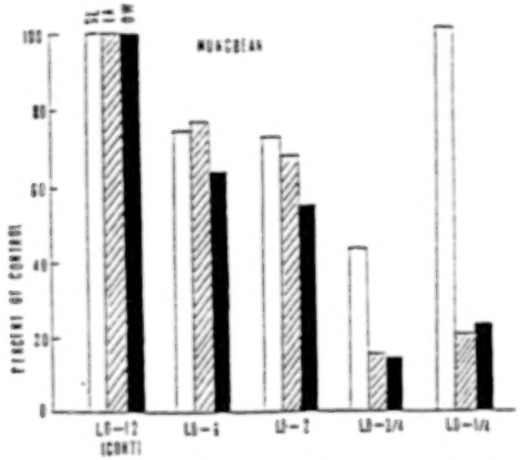

Fig. 3. Growth responses to different regimes of light-dark cycles for three weeks after sowing. SL:vine length, LA:leaf area, DW:dry weight.

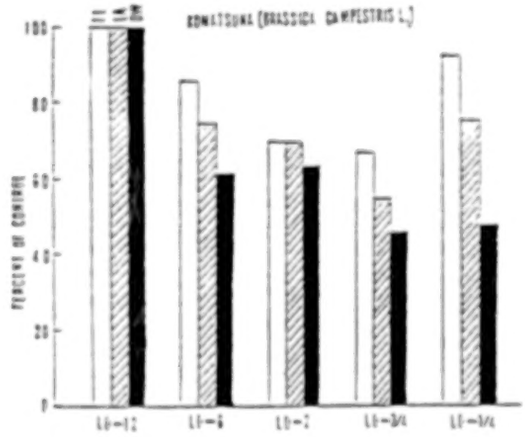

Fig. 4. Growth responses of Komatsuna plants to different regimes of light-dark cycles for 4 weeks after sowing. LL:maximum leaf length, LA:leaf area, DW:dry weight.

Shortening of the cycles gradually decreased the chlorophyll content in leaf blades, whereas it increased the a/b ratio in both species (Table 1). This suggests that reduced photosynthetic activity is caused by a decrease in the total chlorophyll and chlorophyll b. With shortening cycles, leaf diffusion resistance increased, whereas the transpiration rate tended to decrease. In 90- and 30-minute cycles, the failure of stomatal function was suggested from the values of diffusion resistance and transpiration rate. These responses also indicate that such short light-dark cycles inhibits the carbon dioxide exchange rate in leaves (Table 2). Conclusively, plant growth in a 90-minute cycle of light-dark period, as in the flight of space shuttle, may be strikingly retarded /2/, because of the inhibition of photosynthesis and transpiration. Further studies are needed to find more normal growth under a variety of lighting regimes of longer duration or shorter cyclic period in minutes or seconds /1/.

Table 1. Chlorophyll content chlorophyll a/b ratio in leaves as affected by the lengths of a light-dark cycle.

Light regime	Mungbean *		Komatsuna **	
	Chl a+b (mg dm^{-2})	a/b	Chl a+b (mg dm^{-2})	a/b
LD-12	2.20^{a}	2.46^{a}	3.29^{a}	2.46^{a}
LD-6	1.80^{b}	2.54^{ab}	3.03^{a}	2.65^{b}
LD-2	1.97^{b}	2.70^{bc}	3.09^{a}	2.71^{b}
LD-3/4	1.44^{c}	2.36^{a}	2.54^{b}	2.78^{b}
LD-1/2	0.75^{d}	2.87^{c}	1.36^{c}	3.07^{c}

Note : * determined on 23rd day after sowing by use of full-grown leaves. ** determined on 29th day after sowing by use of the largest leaves.

Different letters on the shoulder of the mean value show the significant difference for the multiple range test at the 5-percent level.

Table 2. Diffusion resistance and transpiration rate of leaves as affected by the length of a light-dark cycle.

Light regime	In light(L)		In darkness(D)		L/D ratio of	
	DR (s cm^{-1})	TR (μg cm^{-2}s^{-1})	DR (s cm^{-1})	TR (μg cm^{-2}s^{-1})	DR	TR
Mungbean*						
LD-12	2.72^{a}	5.53^{a}	9.76^{a}	1.48^{a}	0.28	3.74
LD-6	3.18^{a}	5.08^{a}	9.57^{ab}	1.79^{a}	0.33	2.84
LD-2	4.16^{a}	3.42^{a}	12.46^{b}	0.95^{a}	0.33	3.59
LD-3/4	10.92^{b}	1.22^{b}	10.30^{ab}	1.20^{a}	1.06	1.02
LD-1/2	11.41^{b}	1.38^{b}	13.23^{ab}	1.30^{a}	0.86	1.06
Komatsuna**						
LD-12	1.14^{a}	10.36^{ab}	6.94^{a}	2.11^{a}	0.16	4.91
LD-6	1.11^{a}	9.73^{a}	6.13^{a}	2.82^{a}	0.18	3.45
LD-2	1.05^{a}	7.88^{b}	2.77^{ab}	3.93^{ab}	0.38	2.01
LD-3/4	0.99^{a}	8.89^{ab}	1.61^{b}	6.16^{b}	0.61	1.44
LD-1/2	1.29^{b}	3.78^{c}	4.36^{a}	3.05^{a}	0.75	1.24

Note : * determined on the abaxial side of leaf on 22nd day after sowing. ** determined on the abaxial side of full-grown leaf on 19th day after sowing.

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